British Journal of Dermatology

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PL 0173/0036 PA 278/8/1

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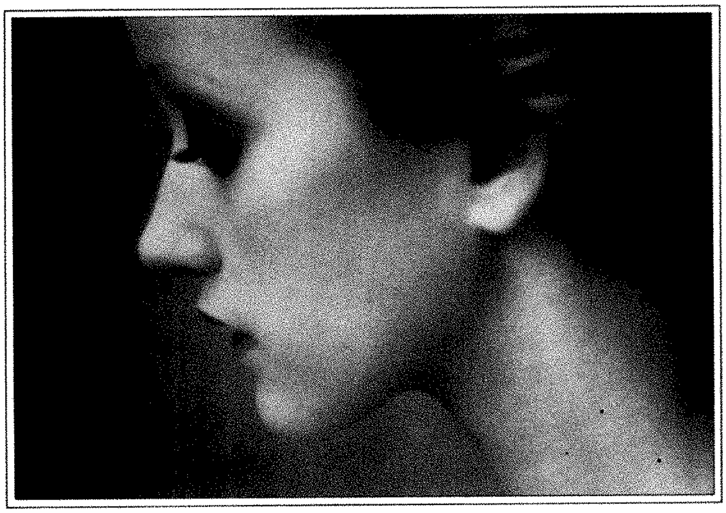

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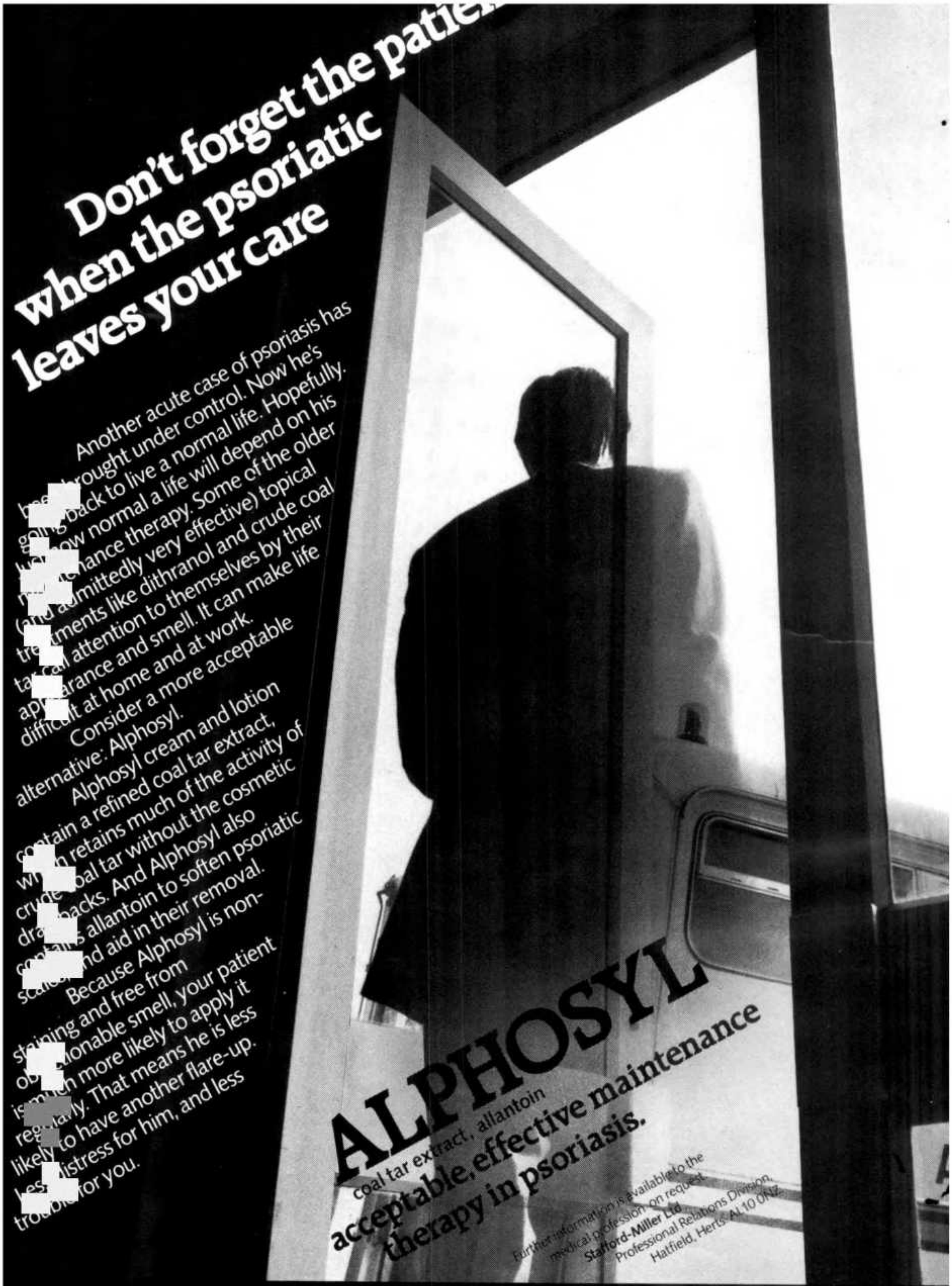

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when the psoriatic leaves your care

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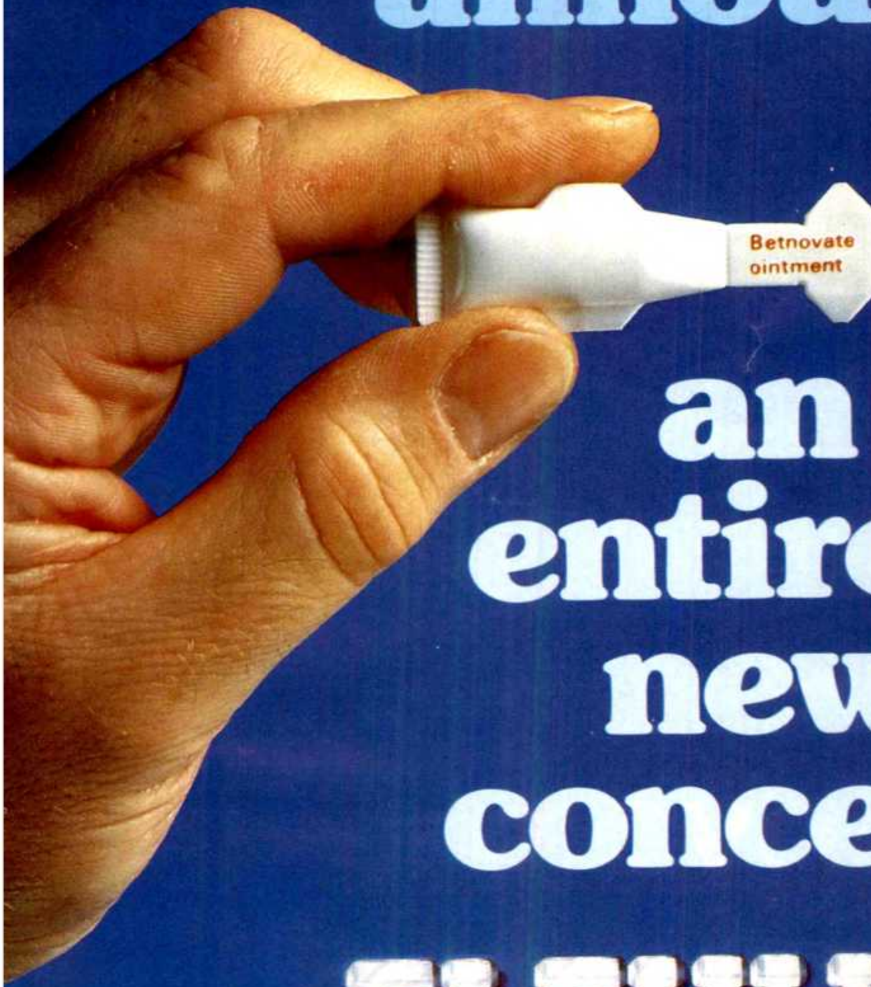

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Prescribing information

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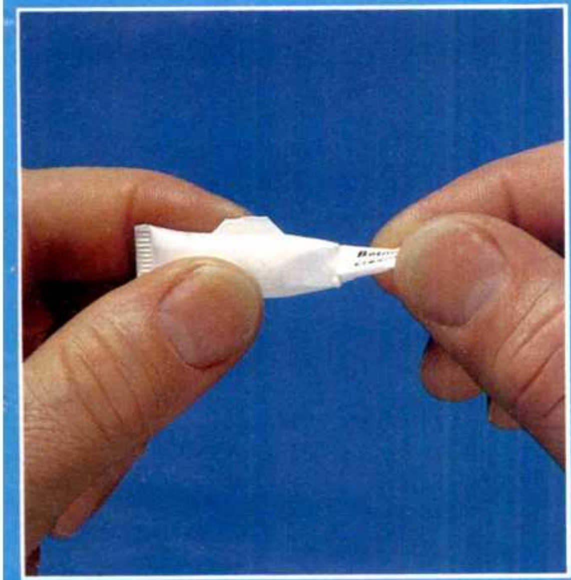
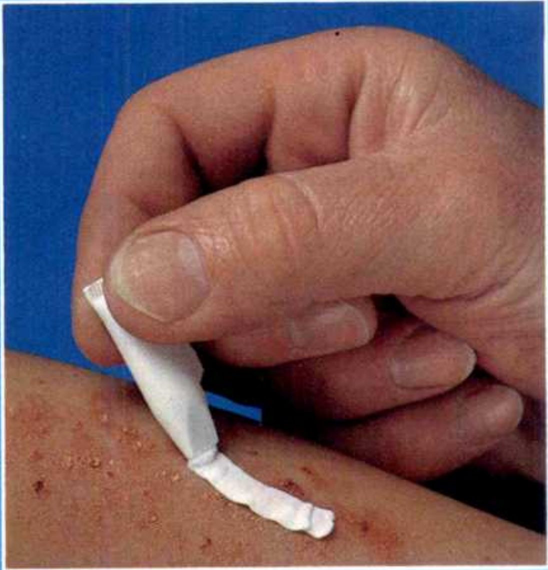
Side effects
General:
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also, prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit

A new measure of control in topical steroid therapy





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Betnovate, Dermovate, Eumovate, Efcortelan and Flexules are trade marks. Further information is available from:
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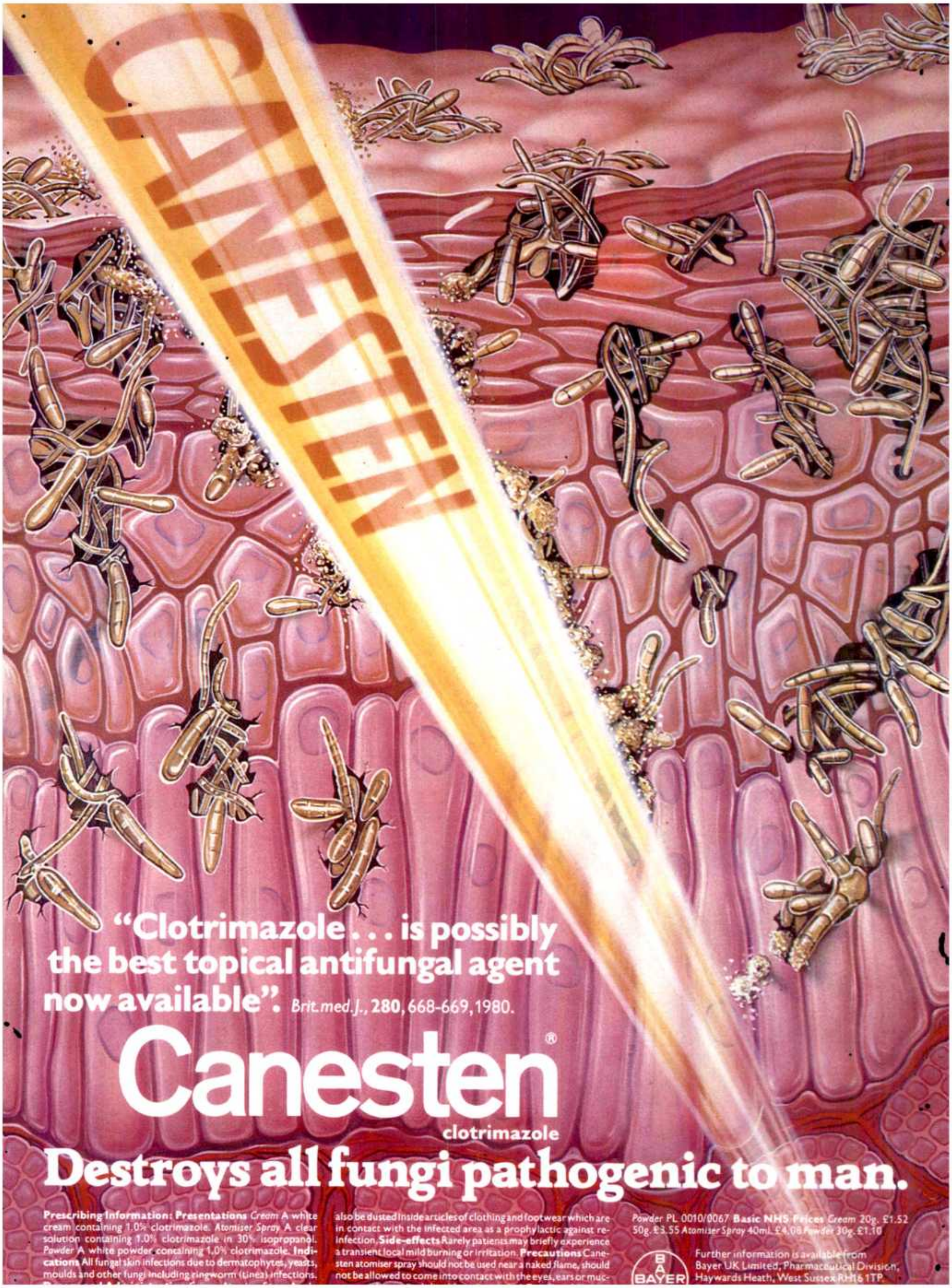

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BAYER

Prescribing Information: Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powder containing 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections.

also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection. **Side-effects** Rarely patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a naked flame, should not be allowed to come into contact with the eyes, ears or muc-

Powder PL 0010/0067 **Basic NHS Prices** *Cream* 20g. £1.52 50g. £3.55 *Atomiser Spray* 40ml. £4.08 *Powder* 30g. £1.10

Further information is available from
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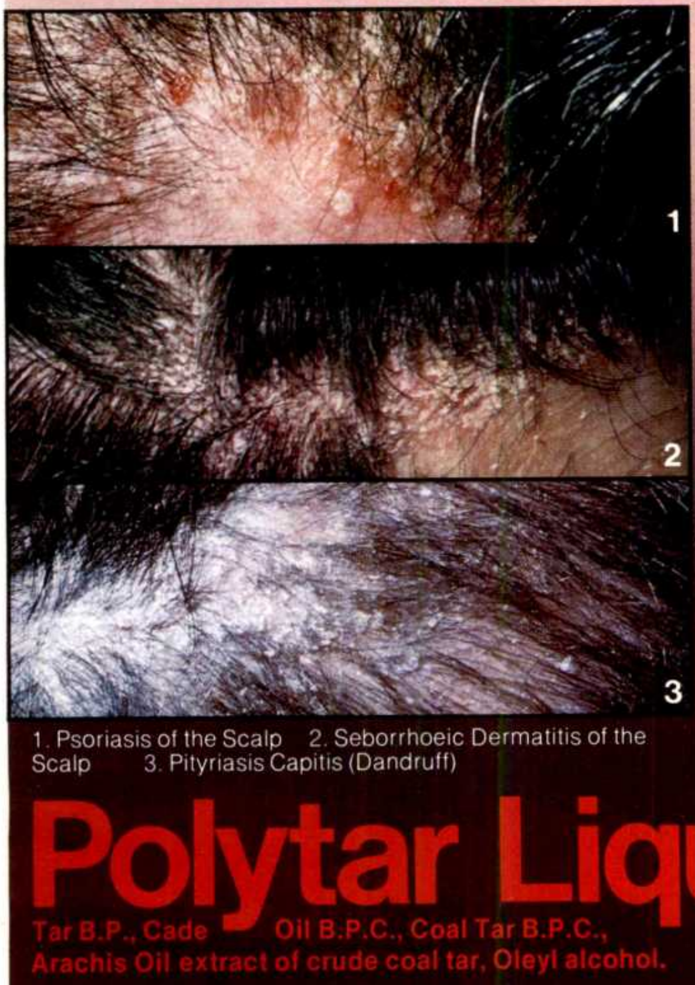
They all have one thing in common — the treatment



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Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
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	1 litre	£3.90
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formation

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lourless evaporative
lution containing
% w/v aluminium
loride hexahydrate.
DICATION:
r the topical treat-
ent of hyperhidrosis
ecifically involving
e axillae, hands or
et.
RECTIONS FOR USE:
pply to the affected
es at night as
quired and allow to
y. Wash off in the
rning.

PRECAUTIONS
Restrict application t
affected sites only.
Keep away from the eyes
PACKAGE QUANTIT
AND BASIC NHS PRICE
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bottle £2.25
REFERENCE
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Journal, 1978, 2, 84
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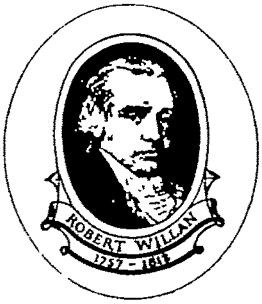

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Clinical and Laboratory Investigations

Clinical and photobiological characteristics of Japanese xeroderma pigmentosum variant*

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SUMMARY

Clinical and photobiological differences between Japanese patients belonging to xeroderma pigmentosum (XP) variant and complementation group A were studied, especially focussing on XP variants. All of ten XP variant patients commonly manifested a delayed onset of pigmented freckles as the initial symptom around 5–7 years old without acute sun erythema, in contrast to the early manifestation of acute solar erythema during infancy in XP group A patients. Six XP variant patients tested showed normal and three showed low minimal erythema doses (MEDs), at the 24 h reaction peak after monochromatic u.v. (280–330 nm) irradiation, while XP group A patients had definitely low MEDs (280–350 nm) with abnormally delayed peaking of the erythema reaction at 72 h. In cell culture studies, all XP variant strains exhibited normal levels of 254 nm u.v.-induced, unscheduled DNA synthesis (UDS), 1·4–2 times more accumulation of excision DNA breaks by arabinofuranosyl cytosine and hydroxyurea due to a subtle defect in the later polymerization step of excision repair, and a slightly higher sensitivity to u.v. cell killing than did normal cells. With respect to the synergistic effect of caffeine on u.v. lethality, XP variant strains could be divided into caffeine-susceptible (eight cases) and caffeine-resistant (two cases) subgroups. The extent of excision-break accumulation was greater in the former subgroup than in the latter. All of eight XP variant patients whose cells showed caffeine potentiation of u.v. lethality had already had skin malignancies, but two sib patients whose cells were caffeine-resistant had as yet had no neoplasm. It is strongly suggested that in XP variant, caffeine-susceptibility may be related to the development of neoplasms.

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Abbreviations: XP, xeroderma pigmentosum; MED, minimal erythema dose; UDS, unscheduled DNA synthesis; araC, 1-β-D-arabinofuranosyl cytosine; HU, hydroxyurea; BCE, basal cell epithelioma; SCC, squamous cell carcinoma; KA, keratocanthoma; AK, actinic keratosis. The terms XP and XP variant are used to specify the incision-defective and -proficient groups, respectively.

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XP is a rare autosomal recessive disorder characterized by abnormal sun sensitivity of the skin, DNA repair defects and a predisposition to cutaneous malignancies (Robbins *et al.*, 1974; Cleaver & Bootsma, 1975; Pawsey *et al.*, 1979). Cultured cells from XP patients of seven complementation groups A–G all have a defect in the first incision step of nucleotide excision repair of u.v. damage, whereas another group, XP variant, is proficient in that repair step (Bootsma, 1978, 1979; Burk *et al.*, 1971; Cleaver, 1968, 1972; Takebe *et al.*, 1977, 1978; Zelle & Lohman, 1979). The previous studies have shown that XP variant cells exhibit either normal or a slightly greater u.v. sensitivity to cell killing than do normal cells (Arlett *et al.*, 1975; Cleaver, 1972; Fujiwara & Tatsumi, 1976; Lehmann *et al.*, 1975; Maher *et al.*, 1975), caffeine potentiation of u.v. cell killing (Arlett *et al.*, 1975; Fujiwara & Tatsumi, 1976; Lehmann *et al.*, 1977; Maher *et al.*, 1975, 1976), decreased host-cell reactivation (Day, 1974; Takebe *et al.*, 1977), and a possible fault in 'post-replication repair' (Lehmann *et al.*, 1975, 1977) as a biochemical criterion for the diagnosis of XP variant. However, several recent results have provided a different interpretation, such that XP variant cells may have a defect in the later polymerization/ligation step of excision repair (Cleaver *et al.*, 1979; Dingman & Kakunaga, 1976; Dunn & Regan, 1979; Fornance *et al.*, 1976; Fujiwara & Satoh, 1979).

Regarding erythema induction with artificial u.v., Ramsay & Giannelli (1975) and Pawsey *et al.* (1979) reported that the skin of XP variant patients exhibited normal erythemal reactions to monochromatic u.v. of sunburn wavelengths, in contrast to the abnormal reaction correlated with repair deficiency in XP patients.

We will focus mainly on the clinical and cellular characteristics of Japanese XP variants. We have collected ten XP variant patients in our University Hospital, and performed skin photosensitivity tests and cellular-molecular studies using cultured cells. We have found differential clinical features, a new phototest result, and a relationship between caffeine susceptibility and cancer proneness in XP variant.

MATERIALS AND METHODS

Skin photosensitivity test

Monochromatic u.v. radiation was obtained from a JASCO Model CRM-FM grating monochromator with a 5 kW xenon arc source (Japan Spectoscopic Co., Ltd., Tokyo). Ultraviolet fluences at various monochromatic wavelengths used were measured by the use of the calibrated JASCO thermopile. Usually unexposed skin of the back of each patient or normal volunteer was exposed to various fluences of monochromatic u.v. radiation at 10 nm intervals between 280 and 360 nm wavelengths. The MED (mJ/cm^2) at each wavelength was determined at 24, 48, and 72 h after irradiation.

Cell strains, media and culture

Fibroblast cultures were derived from skin explants biopsied from the identical inner parts of the upper arms of normal, XP and XP variant subjects. The cell strains used were NHSF3, NHSF6 and NHSF40 from normal subjects (Fujiwara & Tatsumi, 1976; Fujiwara, 1978); XP4 to 6KO strains (Fujiwara, 1978; Fujiwara & Tatsumi, 1976) and XP11KO (this study) from XP group A patients; XP3KO, XP8KO, XP9KO, XP12KO (Fujiwara & Tatsumi, 1976; Fujiwara, 1978) and XP16KO to XP22KO from XP variant patients (this study, see Tables 1–3). Cells were used for experiments between the second and tenth 1:2 split passages.

Cells were grown in Eagle's minimal essential medium (MEM, Flow Laboratories, Inc., Rockville, Maryland) supplemented with 10–15% fetal calf serum (Flow Lab.) in a humidified atmosphere of 5% CO_2 in air, as described previously (Fujiwara & Tatsumi, 1976; Fujiwara, Tatsumi & Sasaki, 1977). Overall plating efficiencies of different strains used at various passages ranged from 7·6 to 29·1%.

TABLE 1. Clinical characteristics of XP variant patients and complementation group A XP patients

Patients	Sex	Consan-guineous marriage	Age of skin biopsy and phototest (years)	Acute sun-sensitivity during infancy or childhood	Onset of symptom	Present cutaneous symptoms except tumours	Skin neoplasms* (Age in years)	Mental and neurological complications
Group A XP patients†								
XP4KO‡	F	—	8	++	3–6 m	severe	—	+
XP5KO‡	F	—	6	++	3–8 m	severe	—	+
XP6KO	M	2nd cousins	3	++	4 m	severe	KA(6)	+
XP11KO	M	2nd cousins	7	++	3–6 m	severe	KA(6), BCEs(8)	+
XP variant patients								
XP3KO	M	—	29	±	5–6 y	severe	BCEs(27), AK(26)	—
XP8KO	M	—	52	—	7 y	moderate	KAs (42, 52)	—
XP9KO	M	2nd cousins	26	—	6 y	moderate	KA (17)	—
XP12KO	M	1st cousins	27	—	7 y	severe	BCEs (11, 27)	—
XP16KO§	M	2nd cousins	42	—	5–6 y	moderate	—	—
XP17KO§	F	2nd cousins	33	Unknown	6 y	moderate	—	—
XP19KO#	F	1st cousins	58	—	10 y	moderate	BCEs(57)	—
XP20KO	M	2nd cousins	29	—	5 y	moderate	BCE(25), SCC(23)	—
XP21KO#	M	1st cousins	50	—	10 y	moderate	BCEs(48), KA(20), AK(50)	—
XP22KO	F	1st cousins	37	Unknown	5 y	moderate	BCE, SCC(37)	—

* BCE, basal cell epithelioma; SCC, squamous cell carcinoma; KA, keratoacanthoma; AK, actinic keratosis tumour. The old histopathological results came from the trace-back survey of the medical records.

‡ Our unpublished data by the cell-fusion study.

Superscript symbols (‡, §, #) indicate three pairs of sib patients.

TABLE 2. Laboratory findings in XP and XP variant patients

Patients and strains	Complementation groups	MEDs, mJ/cm²		U.V. survival		UDS* (% of normal)	Caffeine potentiation of cell killing†	Skin neoplasms
		290 nm	300 nm	*n*	*Do*(J/m²)			
XP4KO‡	A	3·6(1·2)**	3·2(3·2)	1·0	0·5	1·0	–	–
XP5KO‡	A	nt	nt	1·0	0·5	2·3	–	–
XP6KO	A	3·6(0·9)	3·4(2·6)	1·0	0·6	1·7	–	+
XP11KO	A	2·4(2·4)	1·8(0·8)	1·0	0·5	2·3	–	–
XP3KO	Variant		1·7	1·1	3·1	94·5	+	+
XP8KO	Variant		8·4# #	1·0	3·1	100·2	+	+
XP9KO	Variant		2·6	1·3	3·1	101·2	+	+
XP12KO	Variant		nt	1·0	3·8	108·8	+	+
XP16KO§	Variant		10·0# #	1·3	4·0	91·3	–	–
XP17KO§	Variant		5·0	1·3	4·0	105·3	–	–
XP19KO#	Variant		8·5# #	1·2	4·2	98·3	+	+
HP20KO	Variant		11·7# #	1·3	4·2	97·1	+	+
HP21KO#	Variant		7·0	1·3	3·2	115·0	+	+
XP22KO	Variant		7·6	1·1	3·9	84·8	+	+
NHSF3, 6, 40	Normal	9·4±0·24(6·7–14·2)		1·5–2·0	5·0	100	–	–

* UDS was autoradiographically estimated after the 4 h [^{3}H]thymidine labelling of non-S phase cells following 10 J/m² 254 nm u.v. (see Materials and Methods).

† Post-u.v. treatment of cells with caffeine potentiated killing in a dose-dependent manner (Fig. 3; Fujiwara & Tatsumi, 1976; Fujiwara, 1978).

** Numerals in the parentheses indicate the MEDs read at 72 h after monochromatic u.v. irradiation.

Symbols (‡ § #) indicate sib patients (# patients with brownish yellow skin colour).

Complementation groups and skin neoplasms taken from Table 1.

Normal values from the phototest of 18 normal volunteers.

Survival parameters, *n*, and *Do*, were derived from survival curves (see Fig. 3).

nt, not tested.

TABLE 3. Excision breaks accumulated with araC-plus-HU in 254 nm u.v.-irradiated cells

Cell strains		Single strands breaks* ($N \times 10^{-15}$ breaks/g DNA)	% of normal
Normal	NHSF6	4·63 ± 0·43†	100
XP group A	XP6KO	0·255‡	5·5
	XP11KO	0·455‡	9·8
XP variants			
Caffeine-susceptible subgroup§			
	XP3KO	9·47 ± 0·26	205
	XP8KO	9·32‡	201
	XP9KO	9·03 ± 0·11	195
	XP12KO	8·34 ± 0·21	180
	XP19KO	8·80 ± 0·39	190
	XP20KO	9·71 ± 0·19	210
	XP21KO	8·90 ± 0·30	192
	XP22KO	8·67 ± 0·38	187
Caffeine-resistant subgroup§			
	XP16KO	6·24 ± 1·24	138
	XP17KO	6·84 ± 0·69	148

* [^{3}H]cells were u.v.-irradiated with 10 J/m^2 and incubated for 4 h in media containing 2·4 uM araC and 2 mH HU. The reference [^{14}C]cells and such [^{3}H]cells were mixed and lysed, and DNA was centrifuged in alkaline sucrose gradients. Mn and N were calculated, as described in Methods. In the absence of araC-plus-HU during a 4 h post-irradiation incubation, no significant excision breaks were detectable, because of rapid processes. The peak fraction (the 19th top-side) of the sedimentation profile of araC-plus-HU treated caffeine-susceptible variant DNA was only two fractions behind that of treated NHSF6 DNA at the 17th fraction, suggesting that a defect in XP variant is subtle.

† Mean ± SD from three determinations.

‡ Single determination.

§ From Fig. 3 and Table 2.

Ultraviolet (predominantly 254 nm) irradiation and survival assay

Exponentially growing cells were trypsinized (0·1% trypsin solution) and plated in appropriate numbers in duplicated 60 mm Falcon plastic Petri dishes (Division of BioQuest, Oxnard, Calif.), as described previously (Fujiwara & Tatsumi, 1976; Fujiwara *et al.*, 1977). The plates were preincubated for several hours until cells attached, washed once with phosphate-buffered saline, and irradiated with predominantly 254 nm u.v. from a 15 W low-pressure mercury germicidal bulb (Toshiba Electric Co., Tokyo) at an incident intensity of 0·56 J/m^2·sec (Fujiwara *et al.*, 1977). The plates were then incubated for 12–14 days until macroscopically visible colonies with fifty or more cells developed. Survival analysis was done in terms of extrapolation number (*n*) and mean lethal u.v. fluences (*Do*), as described previously (Fujiwara & Tatsumi, 1976; Fujiwara, 1978).

Unscheduled DNA synthesis (UDS)

The procedure has been described in detail elsewhere (Fujiwara *et al.*, 1977). Briefly, cells grown on round coverslips were first incubated for 1 h in 5 μCi/ml of [methyl-^{3}H]thymidine (specific activity, 5 Ci/mmole, The Radiochemical Centre, Amersham, UK), u.v.-irradiated, and reincubated for an additional 4 h in 5 μCi/ml of [^{3}H]thymidine in the presence of 2 mM hydroxyurea(HU) (K & K Laboratories, Inc., Hollywood, Calif.). The labelled cells were processed for autoradiography using a Sakura NR-M2 liquid emulsion and a fixed dark exposure period of 10 days. After development, the number of silver grains on 50–100 lightly labelled, non-S phase nuclei were counted. The average number of grains per cell corrected for the background (0·7–1·7 grains/nucleus) was calculated for a measure of UDS.

Accumulation of excision DNA breaks by arabinofuranosyl cytosine (araC)

Accumulated excision breaks of DNA were detected by a modification of the original method of Dunn & Regan (1979). Briefly, exponentially growing NHSF6, XP group A and XP variant cell strains were prelabelled with 0·1 μCi/ml of [^{3}H]thymidine for 3 days, chased for several hours, and u.v.-irradiated with 0 or 10 J/m^2, followed by an incubation for 4 h in the presence of 2·5 μM araC and 2 mM HU. The cells were lysed and centrifuged in 5–20% (w/v) alkaline sucrose gradients using an SW50·1 rotor of a Beckman Model L5-60 ultracentrifuge at 35000 rpm for 1 h at 20°C, as described previously (Fujiwara & Tatsumi, 1976). Before cell lysis, ^{14}C-labelled, unirradiated cells as internal control reference were mixed with ^{3}H-labelled cells described above.

The number of single strand breaks per 1 g of DNA (= N) was calculated by the equation: $N = 6{\cdot}023 \times 10^{23}$ (1/Mni–1/Mno), where Mno and Mni represent the number average molecular weights of DNA from unirradiated and irradiated cells, respectively.

PATIENTS AND RESULTS

Clinical descriptions and characteristics of Japanese XP variants

Japanese XP variant patients have clinically characteristic features as follows. All XP variant patients manifested no recognizable acute photosensitivity, but XP group A patients had histories of marked acute sun-erythema reactions during early infancy from about 3 months after birth when exposed to sunlight (Fig. 1a; Table 1). Characteristically, Japanese XP variant patients generally manifested the initial, delayed symptoms of small pigmented freckles, slight to moderate dryness and verrucous papules (in some cases) on sun-exposed areas, especially facial skin, around the age of 5–7 years (Table 1). Such mild XP symptoms progressed slowly with advancing age. Even at older ages than those of XP group A patients the majority of XP variant individuals, except a few severe cases, presented moderate symptoms without mental or neurological complications (Figs 1b–d; Table 1).

As for cutaneous neoplasms, XP6KO and XP11KO with severe repair deficiency (Table 2) had their first neoplasms, histologically BCE and KA respectively, at age 6 (Table 1). According to Takebe *et al.* (1977, 1978), 30–40% of Japanese XP group A patients develop skin cancers before the age of 20 years. XP4KO and XP5KO group A patients have as yet developed no neoplasms because they are still too young (Table 1). The youngest age in our XP variant series when skin malignancies were recognized was 11 years (XP12KO variant with BCE) (Table 1). Various types of cutaneous neoplasms such as BCE, SCC, KA, and even pre-malignant actinic keratosis (AK) were histologically diagnosed at various ages between 11 and 57 years old (Table 1). Neoplasms tend to develop at an older age in XP variant than in XP group A (Table 1) (Takebe *et al.*, 1977, 1978). In our XP variant series, the two sib patients (XP16KO, XP17KO) have so far had no neoplasms.

MEDs

The curves of MED against wavelength in both XP group A and variant patients were determined by exposing their skins to monochromatic u.v. radiation, and compared with the curve of average MED for eighteen normal volunteers (age range 8–40 years). The MED curves of normal, XP group A and variant subjects showed the lowest MEDs at 300 nm at 24 h (Fig. 2). One exception was XP9KO variant who showed the lowest 24 h MED at 290 nm (Fig. 2b). This length of time was

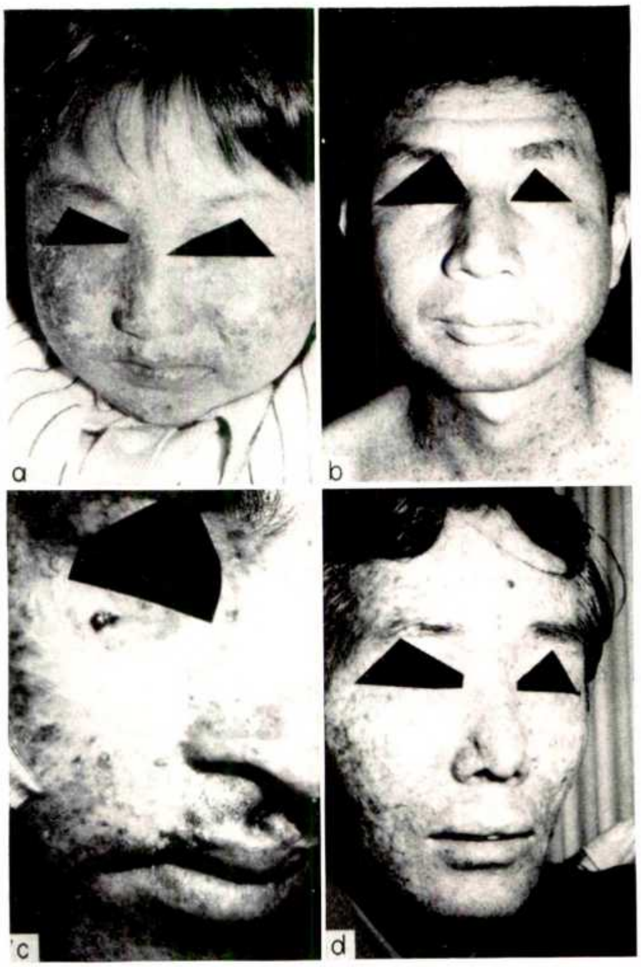

FIGURE 1. (a) Complementation group A XP6KO patient (3-year-old male) exhibiting typical severe XP symptoms. He had acute sunburn erythema at the age of 4 months when exposed to sunlight, followed by dry skin on his face and other exposed areas by 6 months and small pigmented freckles by 1 year old. By the age of 3, he had no neoplasms. Mental retardation was demonstrable by the IQ test (= 60) when 5 years old. Although his face was protected from sunlight by Covermark, his severe XP symptoms were not improved, and at the age of 6 years he developed a small keratoacanthoma. His parents were second cousins. Other laboratory findings are listed in Tables 1 & 2.

(b) XP16KO variant male (42 years) who had no history of acute reactions to sunlight. Small pigmented freckles and dry skin began to appear when he was 5–6 years old. Symptoms progressed slowly with age. When examined at the age of 42 years, the patient had numerous small papules which were histopathologically seborrhoeic and actinic keratoses.

(c) XP12KO variant male (27 years) who had no history of acute sun erythema. Small pigmented freckles were first noticed by the age of 7 years. A small nodule, histopathologically BCE, appeared on the nose at age 11, and since then he has had two walnut- and thumb-sized tumours on the nose, which were histopathologically SCEs. These were excised and the skin was grafted. Over several years he has had a recurrent tumour on the grafted skin of the nose and BCEs on the cheek and lip. The other clinical and laboratory findings are shown in Tables 1 & 2.

(d) XP21KO variant male (50 years). He had no acute reactions to sunlight during childhood, and first noticed a small number of pigmented freckles on the face at the age of 10 years. Since then, numerous pigmented and hypopigmented freckles and verrucous papules have appeared on exposed areas. At the age of 48 there were three bean-sized hyperkeratotic nodules, histopathologically BCEs. See Tables 1 & 2.

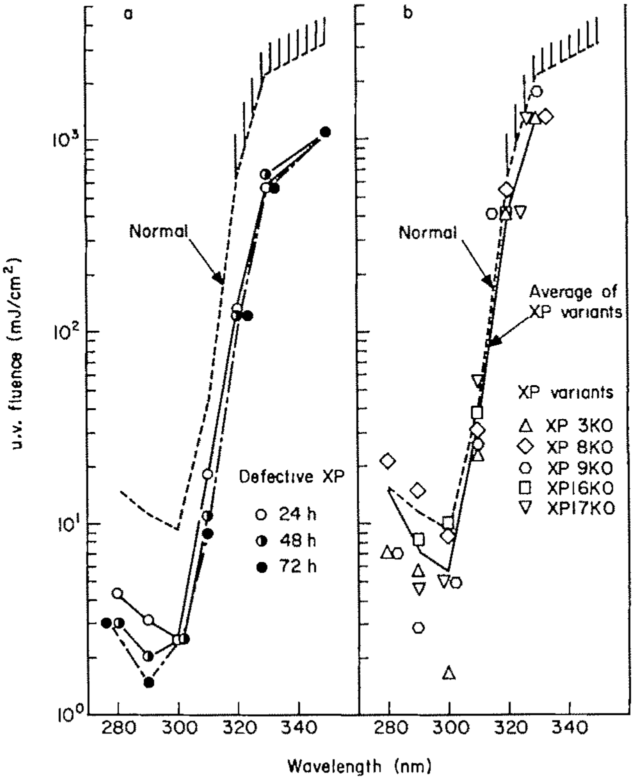

FIGURE 2. MED curves against wavelength. (a) XP group A patients: the time sequence of abnormal erythema at 24, 48 and 72 h showing the average MEDs of three XP patients (XP4KO, XP6KO, XP11KO), compared with the average MED curve of 18 normal subjects (- - -). (b) XP variant patients: the individual MED curves at the 24 h reaction peak of five normally responding and hypersensitive XP variant patients (see symbols), compared with the average MED curve of 18 normal subjects (- - -) and nine XP variants(———).

required for peaking the reaction in normals and XP variant patients. 300 nm MEDs at 24 h in nine XP variants tested varied greatly in a wide range of 1·7–11·7 mJ/cm^2 (Table 2). Since the average MED (300 nm, 24 h) in normal subjects was 9·4±0·24 mJ/cm^2 with a range of 6·7–14·2 mJ/cm^2 (Table 2), three XP variants (XP3, 9 and 17KOs) exhibited lower MEDs than the lower limit of the MED range in normals (Table 2). Thus, these three are referred to as the erythema-sensitive XP variants. Further, the MED curves of these patients are lower than normal between 280 and 330 nm wavelengths (Fig. 2b). However, six other XP variant patients (XP-8, 16, 19, 20, 21 and 22KOs) showed MEDs within normal range, so that these patients cannot be distinguished from normal subjects (Table 2; Fig. 2b).

Figure 2a displays the time sequence of monochromatic u.v. erythema in three XP group A patients (XP-4, 6 and 11KOs) in terms of the average MED. There was an abnormal reaction characterized by delayed peaking 72 h post-irradiation. This observation is in good agreement with the results of Cripps, Ramsey & Ruch (1971), Ramsay & Giannelli (1975), and Pawsey *et al.* (1979). At 72 h, the monochromatic wavelength responsible for the lowest MED in XP group A patients was 290 nm

(Fig. 2a), different from that in XP variant (Fig. 2b). In addition, MED curves over the whole wavelengths at 24, 48 and 72 h in XP group A were significantly lower than in normals (Fig. 2a). However, no XP variant patient showed such a delayed peaking of erythema reaction.

UDS and u.v. survival

Ten XP variant strains exhibited almost normal capacity of UDS (85–115% of NHSFs) after a 4 h [^{3}H]thymidine labelling following 10 J/m^2 254 nm u.v. (Table 2), as described previously (Burk *et al.*, 1971; Cleaver, 1972; Fujiwara & Tatsumi, 1976). In contrast, four XP group A strains showed an extremely low UDS (<3% of normal) (Table 2). Ultraviolet survival parameters, n and Do, in XP variant strains were 1·0–1·3 and 3·1–1–4·2 J/m^2, respectively (Table 2). These values are slightly smaller than those in NHSF strains (n = 1·5–2·0, Do = 5·0–5·5 J/m^2) (Table 2). However, XP group A cells were about 10 times more u.v.-sensitive due to diminished repair capacity (Table 2).

AraC-induced accumulation of endogeneous excision breaks

Excision breaks were measured by alkaline sucrose gradient centrifugation of DNA from cells that had been irradiated with 10 J/m^2 and treated with araC and HU for 4 h. The number of single strand breaks per 1 g of DNA was calculated from sedimentation profiles (not shown). Table 3 shows the average number of accumulated breaks and percentage relative to normal. Accumulation of breaks results from reduced supplies of precursor deoxyribonucleotides and inhibition of DNA polymerases by araC and HU (Dunn & Regan, 1979; Cleaver *et al.*, 1979). The numbers in group A XP6KO and XP11KO cells were less than 10% of normal (NHSF6), as expected from the minimal ability of XP group A cells to incise (Table 2). However, araC and HU-treated XP variant strains accumulated

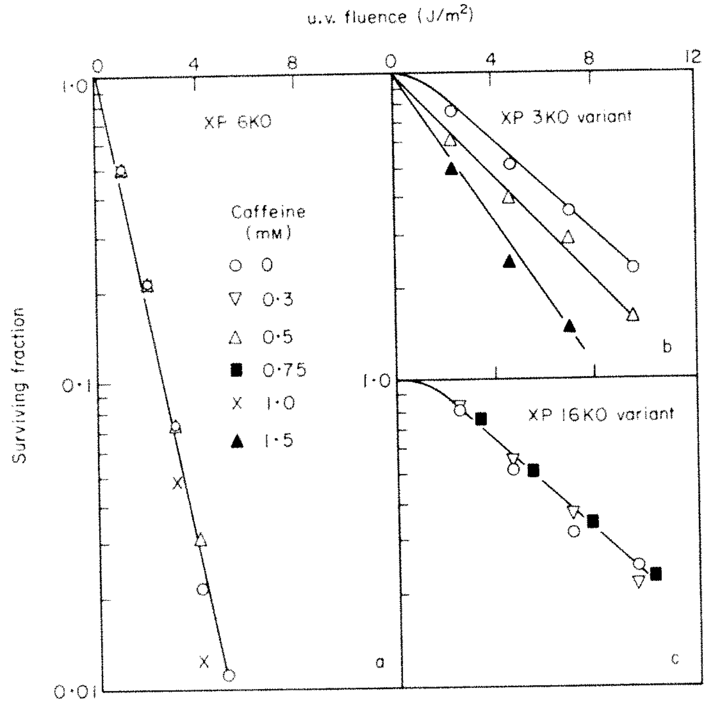

FIGURE 3. Typical 254 nm u.v. survival curves and caffeine effects. (a) caffeine-resistant XP6KO group A cells; (b) caffeine-sensitive XP3KO variant cells; (c) caffeine-resistant XP16KO cells.

1·4–2·0 times more breaks than did NHSF6 cells, indicating together with the results of Dunn and Regan (1979) and Cleaver *et al.* (1979) that XP variant cells may have a defect in the later repair polymerization/ligation step of nucleotide excision repair.

Enhancement of u.v. cell killing by caffeine

The representative survival curves in Fig. 3 show that u.v. killing of XP3KO variant cells is potentiated by caffeine in a concentration-dependent manner, but that of XP6KO variant is not, as described previously (Fujiwara, 1978). We have found such a synergistic effect of caffeine in eight out of ten XP variant patients, but not in cells from XP16KO and XP17KO variant sibs (Fig. 3; Table 2). XP group A strains exhibited no synergistic killing effect of caffeine (Table 2; Fig. 3a), as described previously (Lehmann *et al.*, 1975; 1977; Fujiwara & Tatsumi, 1976; Fujiwara, 1978).

DISCUSSION

Our present results have revealed differential features in clinical studies and skin phototesting as well as in cellular and molecular studies. Clinical and photobiological characteristics of Japanese XP variants are as follows. (1) XP variant patients had no history of acute sun erythema, but XP group A did (Table 1). (2) The delayed onset of the initial small pigmented freckles around 5–7 years old and slow progression of symptoms with age were usual in XP variants (Table 1). XP group A developed severe pigmented freckles, verrucous papules, macules, dryness and pigment abnormalities during childhood. (3) None of the XP variants manifested mental or neurological complications (Table 1), unlike group A, B and D patients (Robbins *et al.*, 1974; Cleaver & Bootsma, 1975; Takebe *et al.* 1977). (4) As compared to the lowest delayed 290 nm MED in group A, the 300 nm MED at the 24 h peak in XP variants was normal or lower than normal (Table 2). This is the first time erythema-sensitive XP variants have been described. XP3KO and XP9KO variants had significantly low MEDs as in group A (Table 2; Fig. 2). (5) Delayed peaking of the erythema reaction, as seen in XPs with severe repair defect, was not observed in XP variants (Fig. 2; Table 2) (Cripps *et al.*, 1971; Ramsey & Giannelli, 1975; Pawsey *et al.*, 1979).

Further, there is no strict correlation between 300 nm MED and 254 nm *Do* in XP variant individuals (Table 2). XP17KO variant patient is XP16KO's younger sister and both culture strains manifest the same *Do* value of 4·0 J/m^2, but their 300 nm MEDs are quite different (Table 2). Possible factors affecting MEDs in XP variants are the skin colour and advanced age, as observed in XP8KO, XP16KO, XP19KO and XP22KO variants (Table 2). Higher melanin content may protect the skin. The photobiological mechanism of the erythema production cannot be adequately explained in molecular terms. The different erythema reaction between XP group A and variant patients suggests a possible link with the difference in DNA repair capacity. The reaction peaks 24 h after irradiation of normal and XP variant subjects, but the peak is delayed to 72 h in XP group A patients with the least incision ability. Thus, it seems that DNA damage, repair defect and repair events play important roles in the production, course and severity of erythema.

The relationship between repair defect, caffeine susceptibility and neoplastic growths in XP variants is considered next. Use of araC and HU, which cause the accumulation of endogenous excision breaks in DNA during the 4 h post-irradiation incubation, has possibly suggested that XP variant cells have a subtle defect in the later polymerization/ligation step of nucleotide excision repair (Table 3), although the first incision step is normal (Table 2). This is more or less compatible with other recent results in XP variants (Dunn & Regan, 1979; Cleaver *et al.*, 1979; Fujiwara & Satoh, 1979). In addition, Fujiwara and Satoh (submitted results) have found that the extent of such a defective polymerization in a caffeine-resistant XP variant is less than in the caffeine-sensitive strains. Here again we have supported such characteristics using the cells from different patients: accumulation of excision breaks by araC and HU appears less in the caffeine-resistant strains of XP16KO and XP17KO sib patients (~140% of normal) than in the seven caffeine-sensitive XP variant strains (180–210% of normal) (Table 3). Clinical manifestations in XP16KO and XP17KO variants (Fig. 1b) are also similar to those in the caffeine-sensitive XP variants (Tables 1 & 2), e.g. XP21KO variant (Fig. 1d). All the XP variant strains show a similar slightly greater u.v. sensitivity (Fig. 3; Table 2), due to a subtle defect in the later repair step as described above. Thus, our present and previous (Fujiwara, 1978) results may imply that the XP variant group consists of caffeine susceptible and resistant subgroups, and that the former condition may accentuate the essential defect in the later polymerization step (Tables 2 & 3).

The pulse-chase studies have shown that the molecular weight of newly synthesized DNA in XP variant cells after u.v. irradiation is much smaller than in unirradiated cells, and afterwards it increases through chain elongation and joining ('postreplication repair') (Lehmann *et al.*, 1975; Fujiwara & Tatsumi, 1976). However, the kinetics of Mw increase as a function of chase time in both normal and XP variant cells are similar to those in unirradiated counterparts, keeping the initial Mw difference (Lehmann *et al.*, 1975; Park & Cleaver, 1979; Fujiwara & Satoh, 1979). Therefore, there seems to be no obvious defect in the ability of postreplication repair in XP variant cells in the absence of caffeine (Park & Cleaver, 1979). However, caffeine causes a long-lived block to a part, but not all, of replication forks and inhibits the slow recovery of post-u.v. DNA synthesis in XP variant cells (Park & Cleaver, 1979; Fujiwara & Satoh; submitted results), thus resulting in the synergistic killing of caffeine-susceptible XP variants. A subtle, but greater defect in repair polymerization in the caffeine-susceptible subgroup than in the resistant subgroup (see above) may produce a higher error-rate, since Maher *et al.* (1975; 1976) indicated that the former XP variant cells produced a higher mutation frequency after u.v. irradiation than did normal cells. As with Fujiwara's (1978) results, Table 2 indicates an excellent correlation, in that all the XP variant patients with caffeine-susceptibility had various types of cutaneous neoplasms (eight out of ten cases). But, the XP16KO and XP17KO sib patients with a caffeine-resistance had no tumours (Table 1).

In conclusion, our study suggests that XP variant cells have a subtle defect in repair polymerization which may be related to the greater caffeine-susceptibility and cancer proneness in XP variant.

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Histiocytosis X—an immune deficiency disease? Studies on antibody-dependent monocyte-mediated cytotoxicity

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SUMMARY

Six children with histiocytosis X, all in clinical remission, were investigated for antibody-dependent cytotoxicity mediated by monocytes and neutrophils. Monocytes demonstrated a reduced cytotoxicity and a normal Fc receptor activity. Judged by light microscopy the monocytes were normal. Pre-incubation with the patient's serum did not influence the cytotoxicity of normal monocytes. Neutrophils from patients with histiocytosis X showed a normal cytotoxic activity. We postulate a functional defect of the mononuclear phagocyte system in histiocytosis X.

The term histiocytosis X (HX) was used by Lichtenstein (1953) to embrace three previously described conditions: Letterer-Siwe disease, eosinophilic granuloma, and Hand-Schüller-Christian syndrome. The essential unity of HX has since been supported by ultrastructural studies (Basset & Nezelof, 1969). By introducing the term histiocytosis X it was stressed that the disease involved proliferation of certain histiocyte-like cells. In view of their enzyme-histochemical characteristics, properties of glass adherence and phagocytosis, and the presence of complement and Fc receptors (Nezelof, Basset & Rousseau-Merck, 1973; Nezelof, Diebold & Rousseau-Merck, 1977; the HX cells are regarded as belonging to the mononuclear phagocyte system. Since HX cells share numerous characteristics with the epidermal Langerhans cell it has been suggested that HX represents a pathological proliferation of Langerhans cells (Corrin & Basset, 1979).

These studies have been carried out on HX cells from tissue lesions. The functional capacity of the constituent cells of the mononuclear phagocyte system, however, has not been studied in HX. We have therefore investigated the function of the circulating component of this system, the blood monocyte, in terms of antibody-dependent cell-mediated cytotoxicity (ADCC). Parallel investigations were performed on neutrophils.

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TABLE I. Clinical information on patients with histiocytosis X

Patient	Age at onset (years)	Organ system involvement	Previous therapy	Age at time of study (years)	Present condition	Present therapy
1. DL	2/12	Skin, lungs, lymph nodes, ears	Vincristine, Prednisone, Topical nitrogen mustard	28/12	Remission for 1 month	Prednisone 2·5 mg daily, topical nitrogen mustard
2. LA	6/12	Skin	Topical steroids	5	Remission for 4 years	None
3. KK	13/12	Skin, gingival mucosa, pituitary, lymph nodes, ears	Vincristine, Prednisone, Topical nitrogen mustard, Vasopressin	4	Remission for 1 year	Vasopressin
4 DH	8/12	Skin, gingival mucosa, ears, temporal bone	Vincristine, Prednisone, irradiation	4	Remission for 1 year	None
5. CJ	3/12	Skin, gingival mucosa	Topical steroids	6	Remission for 4 years	None
6. BC	3/12	Skin, lymph nodes, parietal bone	Vincristine, Prednisone	6	Remission for 4 years	None

MATERIALS AND METHODS

Six children suffering from HX were included in the study. Clinical data are shown in Table 1. The diagnosis was based on the histology of skin biopsies. Only one of the children was receiving immunosuppressive therapy at the time of study. Thirteen children (four girls and nine boys) with an age distribution similar to the HX patients served as controls. These children were admitted to the Department of Pediatrics, Aarhus Kommunehospital, with diseases not involving the immune system and were receiving no drugs.

Monocytes and neutrophils

Venous blood (15 ml) was drawn and mixed with heparin, 20 iu/ml. Monocytes were isolated as previously described (Kragballe, Ellegaard & Herlin, 1980a). Briefly, mononuclear cells were obtained by density gradient centrifugation on Ficoll-Isopaque. After incubation of mononuclear cells in tissue culture flasks at 37°C for 60 min, the non-adherent lymphocytes were washed off. The adherent monocytes were then detached by lowering the temperature to 4°C for 30 min. Neutrophils were recovered as previously described (Beck-Nielsen *et al.*, 1977). Monocytes and neutrophils were identified in cytocentrifuged cell preparations stained for non-specific esterase activity (Leder, 1967). For monocytes, the purity ranged from 89 to 96% in HX patients and from 88 to 97% in controls. For neutrophils, a purity higher than 95% was obtained in HX patients and controls. Judged by the trypan blue exclusion test the viability was invariably higher than 95%.

Antibody-dependent cytotoxicity

The assay was performed as previously described (Kragballe, Ellegaard & Herlin, 1980b). Human type B Rhesus-negative erythrocytes served as target cells. Hyperimmune human anti-B serum was used as anti-target cell antibody. In this assay monocytes and neutrophils, but not lymphocytes, are effector cells. Tests were set up in duplicate. To 100 μl monocytes or neutrophils (1·0 × 10^6/ml) were added 100 μl ^{51}Cr-labelled erythrocytes (1·0–8.0 × 10^6/ml) and 100 μl antiserum in a dilution inducing maximal lysis (1 : 10). In control tubes monocytes and antiserum were replaced by non-labelled erythrocytes (1·0 × 10^6/ml) and pooled AB serum, respectively. After centrifugation at 150 g for 1 min the tubes were incubated at 37°C for 1 h or 18 h. Then half of the supernatant (S) was withdrawn and counted together with residues (R) in a gammacounter. The ^{51}Cr-release was calculated according to the following formula:

$$^{51}\text{Cr-release} = \frac{\text{S c.p.m.} \times 2}{\text{S c.p.m.} + \text{R c.p.m.}}$$

By subtracting values for control tubes the specific release was defined. Results were expressed as the number of target cells lysed per monocyte:

$$\frac{\text{Number of target cells} \times \text{Specific release}}{\text{Number of monocytes}}$$

Cytotoxicity inhibition studies

Fresh serum from the HX patients was studied for the ability to inhibit cytotoxicity of normal monocytes. Normal monocytes were incubated at 37°C for 30 min with 25% (v/v) serum from HX patients or normal AB serum. After four cell washings the cytotoxicity assay was performed.

Monocyte binding of IgG-sensitized erythrocytes

The ability of monocytes to form rosettes with erythrocytes coated with IgG was determined as described previously (Kragballe, Herlin & Jensen, 1981).

Statistical analysis

A Wilcoxon test for two samples or pair differences was used for statistical evaluation. A P value below 0·05 was considered significant.

RESULTS

The clinical data on HX patients are shown in Table 1. The disease started at the age of less than 15 months. Judged from clinical and radiographic criteria two or more organ systems had been involved in most patients, but one patient had only had signs of skin involvement. Four of the six patients prior to the study had received systemic therapy with vincristine and prednisone, but only one had received therapy within the last year. At the time of study all patients were in clinical remission.

The children who served as controls showed the same monocyte and neutrophil cytotoxicity as healthy adults (Kragballe *et al.*, 1980b). Monocyte-mediated cytotoxicity was reduced in all six patients ($P<0{\cdot}01$) (Fig. 1). The results shown were obtained at a target to monocyte ratio of 8 : 1 after incubation for 18 h. But the same degree of cytotoxicity reduction was found at different target cell to monocyte ratios (1 : 1–8 : 1) and at different periods of incubation (1 or 18 h) (data not shown). In five of the six HX patients, neutrophil cytotoxicity was determined and found to be normal (Fig. 1).

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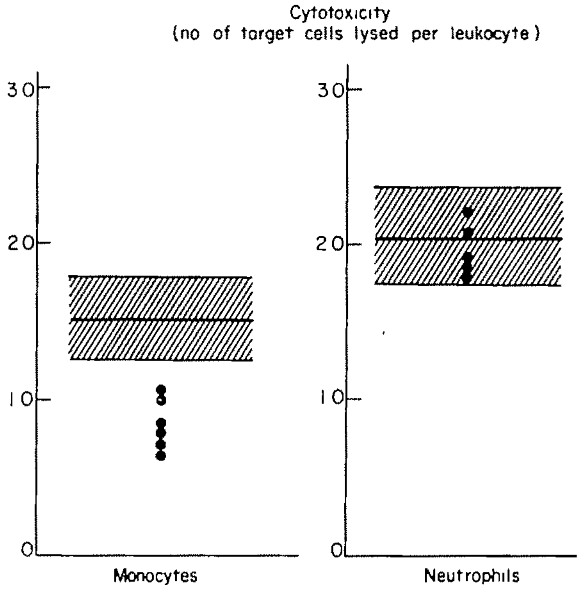

FIGURE 1. Antibody-dependent cytotoxicity mediated by monocytes (n = 6) and neutrophils (n = 5) in histiocytosis X. The horizontal line represents the median value and the shading the 95% confidence limits for normals as determined in thirteen children. The results shown were obtained at a target cell to leukocyte ratio of 8: 1 after incubation for 18 h.

The proportion of monocytes from HX patients forming rosettes with IgG coated erythrocytes did not deviate from that of controls (90–96% and 91–96%, respectively). Sera from HX patients were without effect on the cytotoxicity of normal monocytes. By light microscopy, nuclear morphology and cytoplasmic esterase activity of monocytes were the same in HX patients and controls.

DISCUSSION

In children suffering from HX, but in clinical remission, monocytes, in contrast to neutrophils, exhibited decreased cytotoxicity. Our results suggest that HX is a disorder of the mononuclear phagocyte system and indicate a persisting functional abnormality in this cell lineage.

All our six patients were suffering from HX as proven by classical findings in skin biopsies. The young age of the patients, the clinical presentation with skin lesions, and the absence of skeletal involvement, justify classification of four of the patients as Letterer-Siwe type. Although the two other patients had slight bone involvement this finding is still compatible with the diagnosis of Letterer-Siwe type, especially because the skin lesions were prominent. Since Letterer-Siwe disease constitutes only one (the most disseminated) variant of HX, our results are necessarily not representative for the whole group of HX patients. However, the benign course of HX in our patients does not suggest that impaired monocyte function is associated with a bad prognosis.

The high incidence of terminal infection in HX patients coupled with occasional reports of the simulation of Letterer-Siwe disease in patients with primary immune deficiency (Cederbaum *et al.*, 1974), have pointed to a possible underlying immune defect in these patients. However, normal lymphocyte functions have been reported in HX patients with active disease (Leikin *et al.*, 1973) and during remission (Thommesen, Frederiksen & Jørgensen, 1978). Recently, Osband & Parkmann (1978) reported that blood mononuclear cells from HX patients reacted against different autologous cells *in vitro*, and they suggested that HX might be an autoimmune disorder.

Because HX cells not only morphologically but also functionally share characteristics with the mononuclear phagocytes, it was relevant to study blood monocytes. The presence of normal Fc receptor activity and the absence of inhibiting serum factors indicated that an intracellular defect is underlying the decreased monocyte cytotoxicity although other properties of the monocyte might be preserved. The reported rise in the fraction of mononuclear phagocytes in the bone marrow of HX patients (Feldges *et al.*, 1978) might be another manifestation of abnormalities within the mononuclear phagocyte system in this disease. The normal neutrophil function might explain why our patients did not suffer from infections despite defective monocytes.

HX is regarded by most physicians as a neoplastic disorder, but the infiltrate may be inflammatory rather than neoplastic (Daneshbod & Kissane, 1978), the infiltrating cells being well-differentiated and cytologically having a benign appearance (Rappaport, 1966; Cline & Golde, 1973). Our results might support the notion that HX is an immune deficiency disease.

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Trimethylmethoxyphenyl-retinoic acid (Ro 10-1670) inhibits mitogen-induced DNA-synthesis in peripheral blood lymphocytes *in vitro*

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SUMMARY

Trimethylmethoxyphenyl-retinoic acid (TMMP-retinoic acid, Ro 10-1670) is the main metabolite of the aromatic retinoid Ro 10-9359 (Tigason, etretinate), which is now in clinical use. Therefore, we selected this metabolite for our *in vitro* studies on human lymphocytes.

The following findings were obtained: TMMP-retinoic acid is practically insoluble in aqueous solution. It remains partly in solution only if bound to protein. Under these conditions it had no influence on DNA-synthesis of human peripheral blood lymphocytes added *in vitro* without lectins (5–12·5 ng/ml culture medium). However, if human peripheral blood lymphocytes were cultured *in vitro* with lectins (ConA, PHA-M, PHA-P and PWM) and in the presence of TMMP-retinoic acid (25 μg/ml culture medium) no induction of DNA-synthesis was seen. The altered lymphocytic response in presence of TMMP-retinoid *in vitro* was clearly dose-dependent at these levels. Trypan blue exclusion tests showed no evidence of cytotoxicity.

Our studies clearly indicate that TMMP-retinoic acid inhibits the biological response of human blood lymphocytes to lectins *in vitro*, at dosages close to therapeutic levels *in vivo*. They confirm that retinoids may exert distinct effects on dermal cells in addition to their influence on keratinocytes, and suggest that the therapeutic action of the drug may be also related to the altered lymphocytic response.

Natural and synthetic retinoids apparently have a marked effect on blood lymphocytes *in vitro*. Among others, Kensler & Mueller (1978) found that retinoic acid inhibits the co-mitogenic influence of 12-o-tetradecanoylphorbol-13-acetate (TPA) on bovine lymphocytes. Wertz *et al.* (1979) were able to demonstrate a dose-dependent blockade of phytohaemagglutinin + TPA induced thymidine incorporation by 5,6-epoxyretinoic acid.

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In the present study we have examined the question of whether the aromatic retinoid derivative Ro 10-9359, which is now used for treatment in a number of skin diseases (Orfanos 1979, 1980; Orfanos *et al.*, 1979), has similar effects on human lymphocytes *in vitro*. As the free acid trimethyl-methoxyphenyl-retinoid (TMMP-retinoic acid, Ro 10-1670) appears in the blood a few hours after taking the drug, we preferred this substance for our experiments. TMMP-retinoic acid obviously represents the actually effective metabolite of the oral compound (Hänni, 1978).

Our findings revealed that *in vitro* TMMP-retinoic acid inhibits the stimulation of human lymphocytes induced by phytohaemagglutinins (PHA-M, PHA-P), concanavalin A (ConA) and by pokeweed mitogen (PWM) without having any distinct effect on the DNA-synthesis of non-stimulated lymphocytes or being cytotoxic. The drug obviously modifies considerably the lymphocytic response.

MATERIALS AND METHODS

Lymphocytes were obtained by a ficoll-hypaque-gradient from heparinized blood of healthy subjects (30 ml each).

Lymphocyte cultures

Lymphocytes (5×10^5/ml) were cultivated in plastic tubes in RPMI 1640 with 20 mM Hepes, 200 mM L-glutamine, 10% calf serum, 10 μg/ml neomycin and 0·5 U/ml bacitracin in a total volume of 0·5 ml. Each of the non-specific mitogens phytohaemagglutinin PHA-M, PHA-P, concanavalin A and pokeweed mitogen (PWM) were added in the amount of 25μl per test assay; their concentrations were set at maximal stimulation values.

After a 48-h incubation period, 3 μC of ^{3}H-thymidine (New England Nuclear, specific activity 6·7 Ci/mmol) were added per test assay for the next 18 h for radioactive labelling of DNA-synthesis. For processing of the radioactively labelled lymphocytes, 0·5 ml 10% trichloroacetic acid (TCA) was added. After centrifugation, the precipitate was washed three times with 1 ml 5% TCA; before the last washing procedure extraction was done once with 95% methanol, the sediment was taken up in 100 μl 1 N KOH, was transferred to an Aquasol/H_2O scintillator and the radioactivity was measured in a Packard Tricarb.

All reagents used for lymphocyte stimulation were provided by the Seromed Co.

Addition of retinoid Ro 10-1670 (TMMP-retinoic acid) in culture*

Preliminary experiments revealed that TMMP-retinoic acid is practically insoluble in aqueous solution. Quantities of the substance when dissolved in DMSO precipitated immediately after being added to the culture medium RPMI 1640, apparently because the aqueous phase was present in excess. Even with various solutes we did not succeed in maintaining the TMMP-retinoic acid dissolved in culture medium.

For this reason, we incubated the TMMP-retinoic acid in complete culture medium RPMI 1640 for 1 h at 37°C. The samples were then centrifuged for 10 min at 3000 r.p.m., so that non-bound retinoid was separated by sedimentation. The supernatant still contained considerable amounts of the retinoid derivative bound to protein, as shown by different spectra in the 300–400 nm range between loaded and non-loaded culture media. The presence of TMMP-retinoic acid was indicated by an absorption maximum at 340 nm, showing its successful binding to protein. However, precise quantitative analysis of the protein-bound retinoid fraction could not be obtained by this method.

* The free metabolite TMMP-retinoic acid was received as a gift from the Hoffmann-La Roche Company, Basle.

Thus, the data mentioned in this study relate to the originally added amounts of TMMP-retinoic acid and do not correspond to the actually effective amount of the drug.

The culture medium thus enriched with TMMP-retinoic acid was used for our final experiments.

RESULTS

Influence of TMMP-retinoic acid on cultured lymphocytes without addition of mitogens

A possible direct influence of TMMP-retinoic acid on DNA synthesis of non-stimulated lymphocytes was carefully checked. The results are presented in Fig. 1. While phytohaemagglutinin PHA-P alone showed a good stimulation index, no dose-dependent effect of the TMMP-retinoic acid on blast transformation was measurable in concentrations ranging from 5 to 12,500 ng/ml culture medium, when compared with non-stimulated controls.

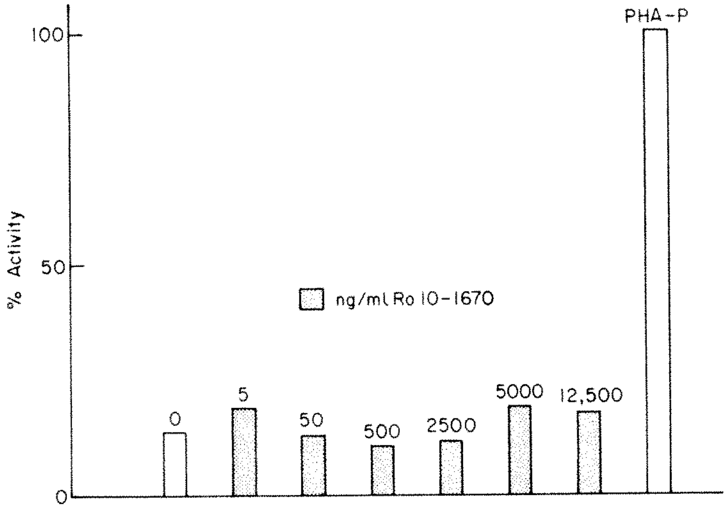

FIGURE 1. TMMP-retinoic acid in concentrations of 5 ng–12·5 μg/ml culture medium has no influence on the DNA-synthesis of peripheral human blood lymphocytes unless mitogen is administered additionally. The columns show the mean value of three determinations.

Influence of TMMP-retinoic acid on cultured lymphocytes with addition of mitogens

Numerous lymphocyte cultures were carried out in retinoid-enriched medium, as described above, with simultaneous addition of mitogens. Figure 2 shows that a clearly inhibiting effect of the TMMP-retinoic acid on lymphocyte proliferation results for the four tested mitogens. Addition of 25 μg TMMP-retinoic acid per ml culture medium inhibited, in its protein bound form, DNA-synthesis. The effect of PHA-P, PHA-M, concanavalin A and PWM was reduced after addition of TMMP-retinoic acid down to 21–34% of the activity without retinoid. Moreover, the mitosis-inhibiting effect of TMMP-retinoic acid *in vitro* was found to be clearly dose-dependent (Fig. 3).

Evaluation of cytotoxicity

Cell vitality was evaluated by trypan dye exclusion in tests at 72 h. No alterations were found in concentrations ranging from 5–25 ng TMMP-retinoid per ml culture medium.

Φ 25,450

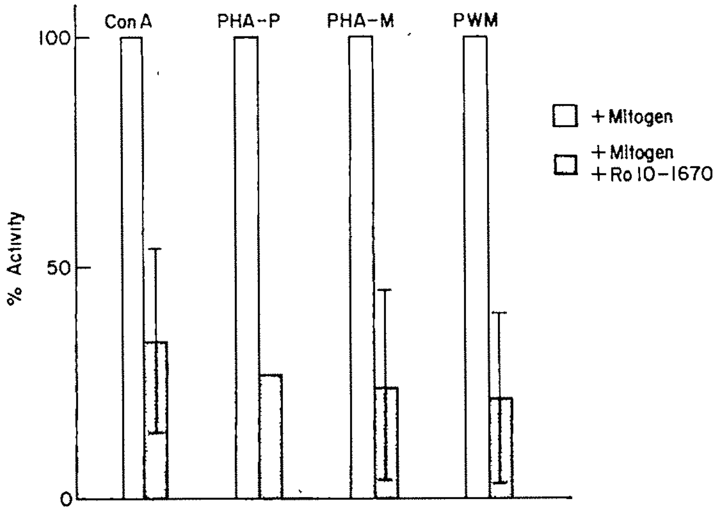

FIGURE 2. Mitosis-inhibiting effect of TMMP-retinoic acid on four tested mitogens. The simultaneous administration of mitogen and 25 μg TMMP-retinoic acid per ml culture medium reduces the DNA-synthesis of cultivated lymphocytes in comparison to control cultures without TMMP-retinoic acid to values between 21–34%. The columns represent the mean value of several cultures: for ConA, n = 6; for PHA-P, n = 1; for PHA-M, n = 7; for PWM, n = 7. Each culture was measured in three-fold assays.

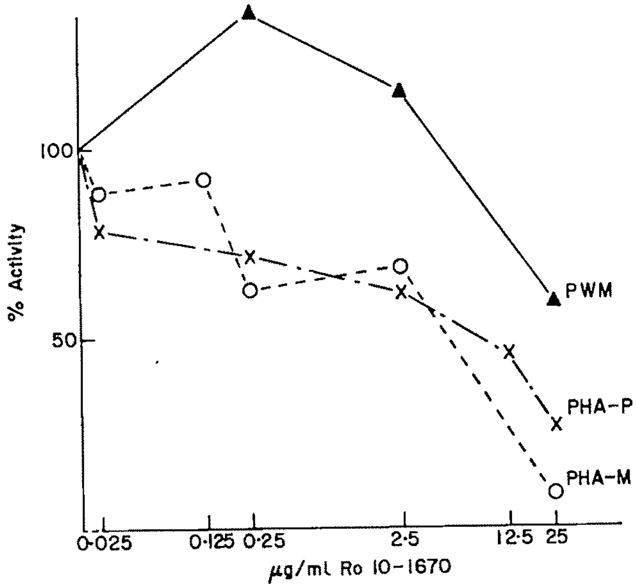

FIGURE 3. Dose dependence of mitotic inhibition by TMMP-retinoic acid for the concentration range of 0·025–25 μg/ml. Between 1 and 2 μg/ml there is some inhibition of DNA-synthesis in cultures with PHA-P and PHA-M. This concentration corresponds to the plasma level at 3 h after oral administration of 2·5 mg/kg Ro 10-9359. Cultures with PWM show no significant change at this concentration level.

DISCUSSION

The findings indicate that, under the influence of the main metabolite of the therapeutically effective aromatic retinoid Ro 10-9359, the TMMP-retinoic acid Ro 10-1670, mitogenic influences on human lymphocytes are inhibited or blocked *in vitro*.

In the presence of this compound at dosages close to or above therapeutic levels, the mitogenic effect of several plant lectins, such as concanavalin A, phytohaemagglutinin PHA-M, PHA-P and pokeweed mitogen, on cultured lymphocytes, is clearly diminished. This indicates that the main oral retinoid metabolite has a distinct effect on human blood cells in terms of inhibiting the mitogen-induced stimulation of DNA-synthesis in human lymphocytes.

A toxic influence of TMMP-retinoic acid can be most likely ruled out, since trypan blue exclusion tests revealed no change in cell vitality. Moreover, at dosages of 75 mg Ro 10-9359 per day *in vivo*, the retinoid plasma level amounts to about 15 μg/ml, provided there is complete absorption of the oral drug; in our *in vitro* experiments we loaded the culture medium with retinoid concentrations at 25 μg/ml culture medium, and only a part of this quantity bound to protein was effectively present in the final experiments. According to Hänni (1978), the plasma level of Ro 10-1670 3 h after oral intake of 2·5 mg/kg of Ro 10-9359 amounts to 1–2 μg/ml, approximately.

The effect of various retinoids on cell growth *in vitro* has so far been investigated on epithelial cells under various conditions: the chemical induction of epithelial carcinomas in skin (Bollag, 1975; Sporn *et al.*, 1976; Weeks *et al.*, 1979), trachea (Sporn *et al.*, 1975), mammary gland (Moon, Grubbs, & Sporn, 1976; Dickens & Sorof, 1979), etc., was inhibited in animal experiments by all-trans retinoic acid and its synthetic derivatives. Various retinoids were also reported to exercise clearly inhibiting influences on the *in vitro* growth of Cloudman melanoma S-91 (Lotan *et al.*, 1978) and on the effects of prostate organ cultures inducible by methylcholanthrene (Lasnitzki & Goodman, 1974). Verma and colleagues showed that the induction of ornithine decarboxylase by 12-o-tetradecanoyl-phorbol-13-acetate can be blocked in the skin of mice by retinoic acid and other retinoids (Verma & Boutwell, 1977; Verma *et al.*, 1978). Also, the deficient growth inhibition caused by cell contact in the case of transformed cells can be restored by retinoic acid (Dion, Blalock & Gifford, 1978). In other recent publications, on the contrary, some synergistic stimulation of DNA synthesis by phorbol esters, polypeptide growth factors and retinoids (Dicker & Rozengurt, 1979), and plant lectins and all-trans retinoic acid (Abb & Deinhardt, 1980) was reported. In the latter publication, low concentrations of retinoid showed some enhancement of PHA-induced mitogenic activity, whereas higher doses revealed marked inhibition.

Thus, most previous reports have been based on animal experiments in which carcinogenic co-mitogens were used in addition to the mitogen additive. In contrast we found an inhibitory effect on thymidine incorporation of human lymphocytes when the mitogenic stimulation was induced by lectins alone and the therapeutically effective retinoid metabolite was used.

Our findings provide evidence that TMMP-retinoic acid added at non-cytotoxic levels *in vitro* may exert an antagonistic effect on mitogenic compounds and block the induction of DNA synthesis in human lymphocytes. Certainly, this effect depends on the biologically available concentration of the drug. The availability of the TMMP-retinoic acid for blood lymphocytes is most likely higher in this *in vitro* system than *in vivo*. To achieve comparable levels, TMMP-retinoic acid was bound to serum proteins; however, the question as to whether our results *in vitro* can be transferred to the situation *in vivo* must remain unanswered. For this reason further experiments with lymphocyte cultures of retinoid-treated patients are required.

Concerning the clinical relevance of these findings, it seems now obvious that aromatic retinoid (via its main metabolite) does exert distinct effects on dermal components (e.g. on the dermal cell infiltrate) in addition to its influence on epidermal cells. In a previous paper from our laboratory we showed that oral retinoid influences cell differentiation inducing the appearance of Sézary-like lymphocytes and of dermal Langerhans cells in psoriatic lesions (Tsambaos & Orfanos, 1980). Together with the *in vitro* findings presented here, we assume that the biological behaviour of lymphocytes may profoundly change in the presence of retinoids. Such compounds seemingly block the action of various mediators on lymphocytes, inhibit mitogenic stimuli, promote cell differentiation into specific cell lines and, therefore, modify considerably the inflammatory and immune response of the cells.

One may speculate that this latter effect of TMMP-retinoic acid has major significance for its action during therapeutic conditions *in vivo*.

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Pr antigens in the skin: distinct localization linked to the stage and the type of keratinocyte differentiation

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SUMMARY

Pr antigens (Pr ags), glycoconjugates of the red blood cell surface, have been traced in stratified squamous epithelia (SSE) with homogeneous and serologically monospecific monoclonal antibodies. Pr ags were expressed either at the plasma membrane level or within the cytoplasm of the keratinocytes (associated with the cytoskeleton). Pr ags were expressed in the basal cells. The distribution within this germinative compartment was in accordance with the concept of a cellular heterogenicity in the basal cell layer. A neuraminidase resistant Pr ag was retained in the maturing compartment of epithelia undergoing parakeratotic differentiation (including psoriatic epidermis).

Monoclonal cold agglutinins may serve as new markers for the study of glycoconjugates in keratinocytes during normal and pathological differentiation.

Cold agglutinins (CA) are auto-antibodies which react optimally at 4°C with red blood cells (RBCs). They are found in high titres in the serum of some patients with virus or mycoplasma infections and in patients with chronic cold agglutinin disease (C.A.D.) (Roelcke, 1974). The great majority of CA are specifically directed against RBC membrane antigens I, i, and Pr complex whose biochemical structure is relatively well known. Moreover the CA developed by patients with C.A.D. are known to be monoclonal antibodies (Roelcke, 1974). The homogeneity of the antibody molecule on the one hand and the relatively precise serological and biochemical definition of their target antigens on the other hand have focused interest on these antibodies as tracers. Such a tool can be used for the investigation of the expression of tissue antigens through the cell differentiation process in various organs (O'Hara, Schumak & Price, 1978; Romer *et al.*, 1979) and in malignant and other pathological states (Feizi, Turberville & Westwood, 1975).

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Although easily obtainable and offering a unique opportunity for the study of cell differentiation and diseases, the skin has not so far been investigated in this field. A preliminary study (Saurat, Didierjean & Habibi, 1980) has shown that Pr antigens were more reproducibly demonstrated in human skin than I and i antigens. The purpose of this paper is to provide evidence that the expression of Pr determinants is related to the stage and to the type of keratinocyte differentiation in epidermis.

MATERIALS AND METHODS

Antibodies

Ten sera with anti-Pr CA were selected for the study. Their specificity within the Pr complex and their agglutination titre at 4°C against 5% suspensions of RBC are listed in Table I. Heat eluates were prepared from sera 1445, 367 and 545. They were incubated at 4°C with protease treated and untreated RBC and studied in parallel by agglutination and indirect immunofluorescence tests (IIF) as described below. The same sera were submitted to six consecutive absorptions on protease treated and untreated RBC and the eluates and absorbates were studied as well in both systems.

For dithiothreitol (DTT) treatment, sera were incubated for 30 min in DTT 0·01M in phosphate buffer pH 7 and tested without further dialysis.

Indirect immunofluorescence tests on stratified squamous epithelia

(1) Tissue specimens from humans, mouse, rabbit and guinea-pig were studied. The specimens were snap frozen in liquid nitrogen, stored at −35°C and processed within a week. Sections 4 μm thick were made in a cryostat microtome at −25°C. Normal human skin was obtained from four blood group A, four blood group O and two blood group B donors. Specimens from involved and uninvolved skin from five psoriatic patients were also studied.

Rabbit lip and oesophagus specimens were processed as previously described. Such a procedure allows the study of orthokeratinized, parakeratinized, mucosal and outer root sheath epithelia in a single lip section: junctional zones between the distinct types of differentiation are also well defined (Saurat *et al.*, 1978). Mouse tail skin specimens were cut parallel to the longitudinal axis in order to obtain sequential zones of parakeratosis and orthokeratosis (Spearman & Hardy, 1977). Mouse and guinea-pig lip specimens were processed similarly to rabbit lip specimens. Guinea-pig ear specimens were also studied.

(2) Indirect immunofluorescence technique (IIF) was performed according to standard techniques as previously described in detail (Saurat *et al.*, 1976). As soon as the cryostat sections were made, the IIF procedure was conducted at +4°C, +20°C and +37°C: incubation for 30 min with the sera containing CA, washing for 30 min in phosphate buffered saline (PBS) pH 7·2, incubation for 30 min with the FITC labelled antisera to light and heavy chains of human immunoglobulins: (goat anti-γ chains of human immunoglobulins (Hyland Laboratories), F/P molar ratio: 2·7; specific Ab concentration: 2 mg/ml; working dilution 1/40; goat anti-μ chains (Hyland) F/P:2·7; Ab: 0·8 mg/ml; working dilution 1/16; goat anti-α chains (Hyland) F/P: 3·1; Ab: 2·1 mg/ml; working dilution: 1/46; rabbit anti-κ chains (Behring) F/P: 2·3; Ab: 1 mg/ml; working dilution: 1/20; rabbit anti-α chains (Behring) F/P: 1·8; Ab: 1 mg/ml; working dilution: 1/20). After washing for 30 min in PBS, they were mounted in buffered glycerol and examined on a Leitz Orthoplan microscope with an optical system for blue narrow band exitation and epi-illumination. Micrographs were taken with high speed Ektachrome 160 ASA.

(3) Immuno-peroxidase technique was performed on 4 μm thick, air-dried sections and incubated with CA for 30 min at their optimal temperature, washed twice for 15 min in PBS, then treated by peroxidase-conjugate for 45 min at room temperature (sheep anti-μ chain of human Ig–Institut

TABLE I. Results of indirect immunofluorescence on normal human skin using human sera containing anti-Pr monoclonal cold agglutinins

			IIF on normal human skin performed at													
Serum		Agglutination	+4°C			+20°C			+37°C			With sera anti-chains				
No.	Specificity	titre	Result	Pattern	Titre	Result	Pattern	Titre	Result	Pattern	Titre	γ	μ	α	κ	λ
1445*	Pr 1 h	16·000	+	B cyt	100	+	B cyt	100	—	—	—	—	+	—	+	—
1705*	Pr 1 h	16·000	+	B cyt	1000	+	B cyt	8000	(±)	B cyt	10	—	+	—	+	—
36771†	Pr 2	2000	+	B cm	200	—	—	0	—	—	0	—	+	—	+	—
36774†	Pr 2	256	+	B cm	10	—	—	0	—	—	0	—	+	—	+	—
545	Pr 2	1000	+	B cm	10	—	—	0	—	—	0	—	+	—	+	—
991	Pr a	128	—	—	0	—	—	0	—	—	0	—	—	—	—	—
1230	Pr ‡	64	—	—	0	—	—	0	—	—	0	—	—	—	—	—
1808	Pr ‡	64	—	—	0	—	—	0	—	—	0	—	—	—	—	—
LEP	Pr a	32	—	—	0	—	—	0	—	—	0	—	—	—	—	—
1178	Pr ‡	32	—	—	0	—	—	0	—	—	0	—	—	—	—	—

B cyt, cytoplasmic staining in basal cells; B cm, staining of the cytoplasmic membrane of basal cells in epithelial crests. * and † two sera from the same patient obtained at different times during the disease course. ‡ Pr subspecificity undefined due to low titre.

TABLE 2. Previous incubation of the skin specimens with papain and trypsin abolished completely the immunofluorescence staining with the four sera (this abolition is shown as a fall in the end point titre of each serum as compared to untreated normal skin). On the contrary both sera 1705 and 1445 (from the same patient) still reacted with neuraminidase-treated skin specimens

			Normal human skin incubated with					
		Normal human skin	Papain*		Trypsin†		Neuraminidase‡	
Serum No.	Specificity	(end point titre)	20°C	37°C	20°C	37°C	20°C	37°C
1705	Pr 1 h	8000	8000	0	8000	0	8000	5000
1445	Pr 1 h	100	100	0	100	0	100	50
367–71	Pr 2	200	200	0	200	0	200	0
545	Pr 2	10	10	0	10	0	10	0

Note that when the substrates were treated at 20°C with the enzymes there was no modification in the antigenic properties (this served as control). The temperature is that of the incubation with the enzymes. The immunological steps were run at the optimal temperature for each cold agglutinin (as shown in Table 1).

* 1 mg/ml 30 min. † 1 mg/ml 30 min. ‡ see Table 3.

TABLE 3. By increasing either the incubation times or the neuraminidase concentration, there was not a decrease but rather an increase in the intensity of the reaction with serum 1705

				Intensity of the staining Normal human skin incubated with neuraminidase									
Serum	Specificity	Dilution	Untreated* normal human skin	30 min at 37°C with				500 U/ml at 37°C for					
No.				5	10	100	500 U/ml	1	5	10	15	30	60 min
1705	Pr 1 h	1·10	+++	+	++	+++	++++	+	+	+	++	++++	++++
367	Pr 2	1·10	+++	—	—	—	—	—	—	—	—	—	—

* incubated in PBS for similar times.

+ and ++++ are arbitrary quotations of the intensity of the immunofluorescent staining as compared to the intensity of the reaction on untreated normal human skin with the serum diluted 1·10.

Pasteur, Paris—containing 1·25 mg of antibody per ml; working dilution: 1/10). Specimens were rinsed twice for 10 min in PBS followed by 10 min in Tris HCl buffer 0·2 M pH 7.6 and incubated in Graham and Karnovsky cytochemical medium for 30 min, again washed in Tris HCl buffer for 10 min then in PBS for 10 min; finally mounted in buffered glycerol.

Immunocytochemical tracing of Pr antigens on isolated keratinocytes

Trypsin isolated guinea-pig ear keratinocytes (Galoppin & Saurat, 1979) were grown for 2 days on coverslips and then air-dried (preliminary experiments have shown that air drying was equal or superior to methanol, acetone, formaldehyde and glutaraldehyde fixation of the cells in preserving Pr antigens for the IIF procedure). After washing with PBS, indirect immunofluorescence technique was undertaken as described above.

Enzymatic treatment of the substrates

Pre-treatment of specimens was performed with trypsin (CNTS Paris, 1 mg/ml PBS buffered), papain (CNTS Paris, 1 mg/ml PBS) and neuraminidase from *Vibrio cholerae* (Behring Paris, 500 units/ml PBS) for 30 min at 37°C or 20°C (as control) before incubation with CA (see Table 3 for variations in concentrations and incubation times).

RESULTS

Expression of Pr antigens in normal human skin (NHS)

(1) Due to lower sensitivity of IIF staining compared to haemagglutination, CA with low titres failed to react by IIF with normal human skin (NHS). Likewise in haemagglutination, positive IIF reactions were shown to be (a) cold dependent, (b) confined to antisera to only one light and one heavy chain, (c) abolished following treatment with DTT (Table 1).

(2) Two IIF patterns were observed in NHS: (a) Serum 1445, anti-Pr 1 h, exhibited a cytoplasmic pattern in outlining black intercellular and nuclear spaces. Most significantly only the cells of the basal layer were stained (basal cytoplasmic pattern B Cyt–Fig. 1a); (b) Sera 367 and 545, anti-Pr 2, showed a membranous pattern with a microgranular appearance. Again the staining was limited to the basal cell layer but only cells clumping within epithelial crests were positive (Fig. 1c).

(3) Eluates and absorbates: the activity responsible for the staining in IIF was found in the eluates from untreated RBC but not in the eluates from RBC treated with papain. Similarly the activity was present in the absorbate from RBC treated with papain but not in the absorbate from untreated red blood cells.

(4) Enzymatic treatment of the substrates: Table 2 shows that the target antigens were destroyed by previous treatment of the human skin specimens by papain and trypsin. Neuraminidase destroyed only the target antigens for anti-Pr 2 sera; the antigen reacting with anti-Pr 1 h serum was not destroyed (Table 3).

Patterns at the cellular level

These were better analysed on isolated cells than on tissue sections. The two different patterns were again seen. The serum reacting with a cell wall membranous pattern on tissue sections (anti-Pr 2) was found to give a patchy staining of the plasma membrane on isolated cells (Fig. 1d). The serum giving an homogeneous cytoplasmic staining on tissue sections (anti-Pr 1 h) was found to give an intra-cytoplasmic filamentous pattern (Fig. 1b).

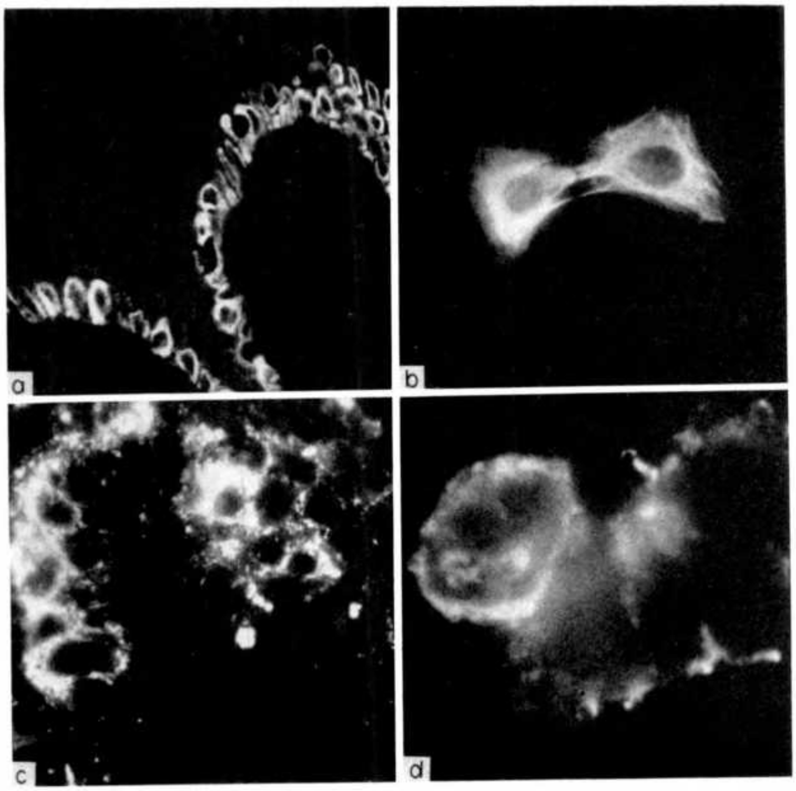

FIGURE 1. (a) Expression of Pr 1 h antigens in normal human skin. Note the cytoplasmic pattern on the basal cells. This cytoplasmic pattern is confirmed by the study on isolated epidermal cells (b). (c) Expression of Pr 2 antigens in normal human skin. Note the microgranular 'membranous' pattern. This pattern is also found on isolated epidermal cells (d).

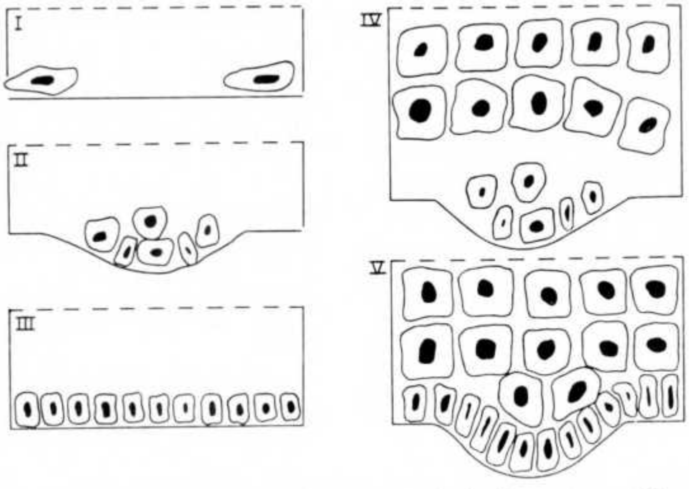

FIGURE 2. Schematic representation of the five types of distribution patterns of Pr antigens within stratified squamous epithelia: Type I, a few scattered cells in the basal cell layer; Type II, basal cells clumping in the epidermal crests; Type III, basal cells homogeneously stained; Type IV, basal cells clumping in the epidermal crests plus upper 'maturing' cells; Type V, diffuse expression in all the epidermis.

Note that Type IV might represent Type II plus expression in the maturing compartment whereas Type V might represent Type III plus expression in the maturing compartment. See Table 4 for correspondence of each type to the differentiation of the epithelium.

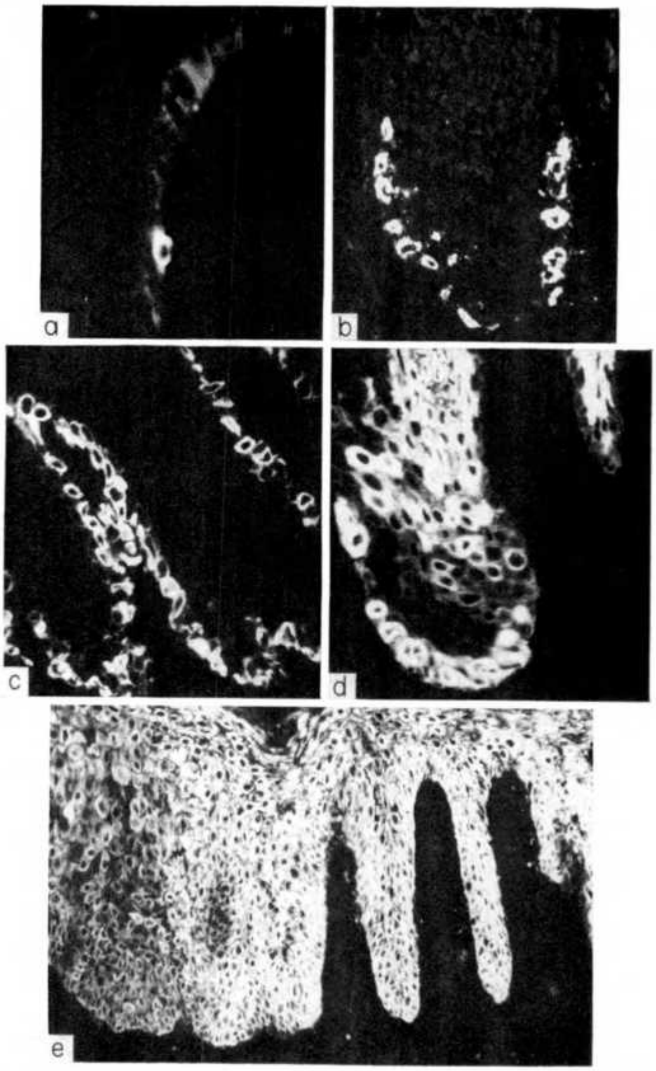

FIGURE 3. The five distribution patterns of Pr antigens within stratified squamous epithelia (see also Fig. 2 and Table 4). (a) Type I = a scattered cell in basal position (rabbit lip—outer root sheath of hairs above the isthmus). This cell is dopa negative and does not incorporate ^{3}HT. Data not shown. (b) Type II = basal cells clumping in the epidermal crests. Rabbit lip (Pr 2). Parakeratotic side. (c) Type III = basal cell layer homogeneously stained. Rabbit lip. Mucous side. (d) Type IV = basal cells clumping in the epidermal crests plus 'upper' staining. (e) Type V = diffuse staining in all the epidermis in human psoriatic skin.

Expression in various types of stratified squamous epithelia (SSE)

Both Pr 2 and Pr 1 h antigens were found to be expressed in the SSE of rabbit, mouse and guinea-pig. The cellular patterns were very similar in the animal tissues to those seen in human skin (i.e. a cytoplasmic staining for Pr 1 h and a cell membrane staining for Pr 2). The distribution patterns at the tissue level were not uniform in all the specimens. Most importantly the localization of Pr antigens was related to the type of differentiation of the epithelium. Figure 2 shows the schematic repre-

TABLE 4. The distribution patterns of Pr 1 h and Pr 2 antigens were not similar. However there was a close relationship between the type of distribution patterns of each antigen and the type of differentiation of the epithelium. Note that, in the mouse, there was a distinct distribution in orthokeratosis and in ORS above the isthmus*

	Orthokeratosis		Mucosa		ORS (above isthmus)*		(ORS (below isthmus*)		Parakeratosis	
	Pr 1 h	Pr 2	Pr 1 h	Pr 2	Pr 1 h	Pr 2	Pr 1 h	Pr 2	Pr 1 h	Pr 2
Human										
Normal	III	II	----	----	I	II	V	III	----	----
Psoriatic (uninvolved)	III	II	----	----	NS	NS	NS	NS	----	----
Psoriatic (lesional)	----	----	----	----	NS	NS	NS	NS	V	II
Guinea-pig										
Ear	III	II	----	----	I	Neg.	V	III	----	----
Lip	III	II	Neg.	Neg.	I	Neg.	V	III	V	Neg.
Rabbit										
Back	III	Neg.	----	----	I	Neg.	V	Neg.	----	----
Lip	III	II	III	III	I	Neg.	V	Neg.	IV	II
Oesophagus	----	----	III	III	----	----	----	----	----	----
Mouse										
Tail	V	Neg.	----	----	V	Neg.	V	Neg.	V	II
Lip	V	Neg.	III	Neg.	V	Neg.	V	Neg.	V	Neg.

ORS, outer root sheath of hairs; Neg., negative. No expression detected; NS, not studied. I–V, type of distribution pattern. See Fig. 2 for significance.

* as determined by serial sections (as previously reported by Saurat & Didierjean, 1980).

sentation of the five distinct distribution patterns. Pr antigens were expressed either in the basal cell layer or in both the basal cell layer and the upper cell layers.

(1) Within the *basal cell layer* three types of localization were observed: (a) only in a few scattered cells (these cells were dopa-negative) (Fig. 3a); (b) only in a group of basal cells clustering in the epidermal crests (Fig. 3b); (c) in all the basal cells (Fig. 3c).

Table 4 shows the close relationships between the type of distribution pattern and the type of differentiation of the epithelium. It also shows that Pr 1 h and Pr 2 antigens were not distributed similarly in the basal cell layer of similar epithelia.

(2) Only Pr 1 h antigens were expressed within the *upper maturing* cell compartment. Such expression in the upper cells was associated either with the expression of Pr 1 h antigens in clustering basal cells in the epidermal crests (Type IV–Fig. 3d) or with the expression in all the basal cells (Type V—Fig. 3e). Again there was a close relationship between the localization of Pr 1 h antigens in the upper maturing cells and the type of differentiation of the epithelium. Such an 'upper' localization was constantly seen in the parakeratotic epithelia including psoriatic human skin and in the outer root sheath of hairs below the isthmus. Pr 2 antigens were not expressed in the upper maturing cells.

Expression in psoriatic epidermis

The expression of Pr 2 antigens was similar in psoriatic skin and in normal skin (i.e. cells clumping in the basal part of epithelial crests). The expression of Pr 1 h was different in the psoriatic skin (Table 4); the antigen was expressed not only in the germinative compartment but also in the maturing compartment. The distribution in the normal skin from psoriatic patients was similar to that in normal human skin.

DISCUSSION

Pr antigen expression in normal human skin

Anti-Pr CA are defined as auto-antibodies reacting optimally at 4°C with RBC membrane antigens which are inactivated by protease treatment. Unlike target antigens for blood group allo-antibodies, Pr determinants are 'public' antigens universally expressed in the human species. On the basis of their serological reactivity with animal RBC, their various sensitivities to different enzymes and their pattern of inactivation by periodate oxidation or carbodiimide treatment of RBC glycoproteins, Roelcke (1973, 1974) has proposed the classification of the Pr complex indicated in Table 5. Haemagglutination inhibition studies of crude or chemically modified RBC glycoproteins have shown that Pr complex determinants are represented by alkali labile oligosaccharide with N acetyl neuraminic acid (NANA) as the terminal amino sugar for Pr 1h, Pr 1d, Pr 2 and Pr 3.

In this study we have demonstrated that Pr 1 h and Pr 2 antigens are expressed in the epidermis. Both are sensitive to trypsin and papain treatment. Only Pr 2 antigens were found to be destroyed by neuraminidase. That Pr 1 h antigens are still detectable in neuraminidase treated epidermal specimens may have several explanations: (a) The sensitivity of epidermal specimens to neuraminidase might be different from that of RBC. This is unlikely because Pr 2 antigens were destroyed after similar procedures on epidermal specimens. (b) The cytoplasmic localization of Pr 1 h antigens in the epidermal cells might modify their sensitivity to neuraminidase; it is known that there are structural requirements for the NANA analogues to serve as substrates for neuraminidase (Suttajit & Winzler, 1971). (c) That the IIF picture may be due to a contaminating antibody with Pr a (neuraminidase resistant) specificity. This was ruled out because eluates from neuraminidase treated RBC did not retain any residual cold agglutinin activity.

TABLE 5. Serological and biochemical identification of Pr subspecificities

	Untreated red blood cells					RBC treated with			Membrane glycoprotein treated with	
							Papain			
	Human	Dog	Guinea-pig	Cat	Sheep	Neuraminidase	Human	Dog	Periodate	Carbodiimide
Pr 1 h	+	—	—	—	—	—	—	—	—	—
Pr 1 d	+	+	+	—	—	—	—	—	—	—
Pr 2	+	+	+	—	—	—	—	+	+	—
Pr 3	+	+	+	+	+	—	—	—	—	+
Pr a	+	—	—	—	—	+	—	—	+	+

+ : antigen present or not inactivated; — : antigen absent or inactivated).

As we were working with homogeneous monoclonal antibodies we can assume that Pr 1 h antigens are neuraminidase resistant within the human keratinocyte.

Distribution patterns and Differentiation

Striking differences in the distribution patterns of Pr 2 and Pr 1 h antigens were found at both the cellular and the tissue levels within the epidermis.

At the cellular level. Pr 2 antigen was found to be expressed on the plasma membrane while Pr 1 h ag was found in the cytoplasm of the keratinocytes. The filamentous pattern of Pr 1 h ag expression suggests that it is linked with the cytoskeleton of the keratinocytes. The fact that closely related glycoconjugates are not found in a similar location within the cell is in accordance with current concepts on the steps of the biogenesis of glycoproteins (Leblond & Benett, 1979).

At the tissue level. It has already been shown that I, i and Pr antigens have distinct distribution patterns in several tissues (Lenhard *et al.*, 1978; Romer *et al.*, 1979), e.g. Pr antigens were expressed in the glomerulus, while I i were found in the proximal tubule of the human kidney. Our findings make two further points: (a) closely related glycoconjugates within a single antigenic system (here the Pr system) have distinct distribution patterns; (b) the distinct distribution pattern is demonstrated within a single cell line (here the keratinocyte) according to its 'stage' during differentiation and according to the 'type' of differentiation toward which the cell is progressing. Stratified squamous epithelia (SSE) provide an appropriate model for studying this second point. Schematically SSE are divided into three compartments (Christophers & Laurence, 1976): (a) The basal germinative compartment in which keratinocytes (called there 'keratoblasts') are able to divide and are considered as being in the less differentiated state; (b) The prickle cell layer and the granular layer in which the cells are maturing (keratocytes); (c) The horny layer, made of fully differentiated cells (corneocytes). Normal 'keratoblasts' may undergo several types of differentiation which are histologically well defined, such as orthokeratotic, parakeratotic and mucosal differentiation.

Clearly distinct expressions of Pr antigens were linked to the different types of differentiation of the 'keratoblast'.

Within the germinative compartment, Pr antigens were expressed either in nearly all the cells or in only some cells. This was reproducible and not related to any kind of technical problem (e.g. the angle of sectioning) because, on similar substrates, all the basal cells were stained with previously described antibodies specific for basal cells (Saurat *et al.*, 1978). Most characteristically the expression of Pr antigens in nearly all rather than in some germinative cells was related to the type of differentiation of the epithelium. For instance Pr 2 ag was expressed in nearly all the germinative cells in rabbit *mucous* epithelia but only in some cells clustering in the epidermal crests in *orthokeratotic* epithelia.

These observations may be interpreted considering the two following concepts: (a) The proliferative (germinative) compartment of the SSE is non-homogeneous in that it consists of several populations of keratinocytes whose kinetic properties are distinct (Potten, 1974; Christophers & Laurence, 1976; Bickenbach & Mackenzie, 1979). (b) The cell kinetics are not similar in epithelia involved in distinct types of differentiation (i.e. orthokeratotic versus mucous, etc. . .) (Gerson *et al.*, 1980).

Therefore the expression of Pr antigens in the basal cell layer might be dependent upon a given stage of the cell within the cell cycle or the initial process of differentiation. Preliminary studies (unpublished data) have shown that the basal cells expressing Pr 1 h antigens were not in S phase as they did not incorporate ^{3}H thymidine. Further work is needed to establish whether or not these monoclonal antibodies may be used for tracing the cell cycle of the epidermal cells.

Another important point from this study was the expression of Pr ag in the maturing compartment of SSE. Pr 2 ags were not expressed in this compartment whereas Pr 1 h ags were expressed at this level in a few types of SSE. With the exception of the mouse, the expression of Pr 1 h ag was linked to a parakeratotic type of maturation. It seems therefore that the expression of Pr 1 h ag is lost as soon as the keratinocyte moves up and progresses in the maturation process, except when the maturation is directed towards parakeratosis. This is strongly supported by the findings in psoriatic patients: Pr 1 h ags were expressed only in the basal germinative compartment in the normal orthokeratotic skin but were also expressed in the maturing compartment of the adjacent lesional parakeratotic skin. Therefore glycoconjugates usually associated with the germinative compartment are retained in the cell maturing toward parakeratinization.

Skin Pr antigens as a target in chronic cold agglutinin disease

Finally the expression of Pr antigens in the keratinocytes has to be regarded in terms of potential targets when high titres of CA are present in the serum. It has been established that CA may have cytotoxic activities in the presence of complement (Pruzanski & Delmage, 1977; Geisen *et al.*, 1976). This activity was dependent on the titre and thermal amplitude of CA. Whether some of the cutaneous symptoms of chronic cold agglutinin disease might be due to any keratinocyte-directed cytotoxicity remains to be established. In a patient with high titres of anti-Pr 1 h CA (Doutre *et al.*, 1978) with large thermal amplitude there was no histological evidence of preferential damage and the direct immunofluorescence was negative in the epidermis. The skin lesions during chronic cold agglutinin disease are thus more likely related to vascular occlusion (Doutre *et al.*, 1978) than to epidermis-directed cytotoxicity.

ACKNOWLEDGMENTS

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Transepidermal chloride flux through hydrated skin: combination chloride electrode

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SUMMARY

A combination chloride electrode was used to determine quantitatively the outward rate of epidermal chloride transport *in vivo*. Regional anatomical variation and the effects of topical agents and cellophane tape strippings on the rate of chloride diffusion were determined. Transepidermal chloride flux through hydrated skin appears to be an alternative method for determining stratum corneum function *in vivo*.

Because the stratum corneum is a major barrier to permeation of many chemicals it presumably would be a rate limiting step for diffusion of electrolytes through the skin. Quantifying transport of electrolytes outward through the skin would seem one reasonable method of measuring barrier integrity, possibly indicating changes or damage to the stratum corneum's barrier before they are visualized clinically or histologically.

Cutaneous electrolyte transport has been measured by two methods:

(1) Using a radioactive tracer to study penetration through the skin by its disappearance or by its appearance inside the body (Tregear, 1966).

(2) Using the non-specific measurement of impedance or resistance across the skin (Kiss & Horvath, 1972). Surface chloride measurements aid in screening for cystic fibrosis (Green, Mascia & Behrendt, 1975) and to evaluate 'epidermal transudate' (Grice *et al.*, 1975).

We used the combination chloride electrode to determine the outward transepidermal flux of chloride through hydrated skin. Regional anatomical variation and the effects of topical agents and of cellophane tape stripping on the rate of diffusion were measured. The chloride diffusion from an anhidrotic skin (burn scar) was measured to assess the contribution of resting level eccrine sweating.

MATERIAL AND METHODS

To determine quantitatively the rate of chloride transport *in vivo* with an electrode requires a fixed volume of solution and a chloride source. A plexiglass block (0·9 × 3·8 × 3·8 cm) with a cylindrical cavity received the electrode and contained the aqueous phase. The block was attached to the skin

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with either silicone cement, or an ECG electrode adhesive; 0·1 mol dm^{-3} sodium nitrate was added to the cavity. The chloride source was the tissue below the stratum corneum and was most probably epidermal interstitial fluid.

A combination electrode (Orion 97-17) and a pH-specific ion meter (Beckman Century SWL or Corning 12) were utilized. The meters had expanded scales from -1400 to $+1400$ mV with uncertainty of ± 1 mV. Six solutions of sodium chloride from $3{\cdot}20 \times 10^{-3}$ to $9{\cdot}98 \times 10^{-3}$ mol dm^{-2} were used to calibrate the electrode; a plot of the log of the concentration versus mV is presented (Fig. 1).

Experimental

The site to be measured was gently clipped if it was hairy and the plastic cup was attached. The cavity was filled with sodium nitrate solution ('Baker analysed' Reagent Grade) 0·4 ml, with a volumetric pipette. The electrode potential was immediately read, recorded, and a stopwatch started. Readings were taken at regular intervals, usually at 5–10 min. In some cases the electrode remained in the cavity for the entire experiment, and in others it was removed and the cavity covered with a glass cover slip between readings. No difference was observed between the two methods. Measurements were generally made on the volar aspect of the forearm; measurements were also made on the palm, the dorsum of the hand, the back, the calf and the dorsal forearm.

Skin Treatment

Measurements were made after treatment with topical agents known to alter the stratum corneum, and after cellophane tape stripping. In the latter, the forearm was stripped with cellophane tape until the surface glistened. The cup was attached to the skin and sodium nitrate solution added. The electrode potential was read for the period listed.

The chemicals, applied using the cups glued to the skin as containers, were dimethylsulphoxide (DMSO) 0·2 ml, acetone 0·2 ml, 2 ml 10% sodium dodecyl sulphate (SDS), 0·2 ml ethyl acetate and a 2 ml distilled water control. Application was followed by 2 min mechanical agitation using a cotton swab. The agents were removed using paper towels or cotton swabs. The sodium nitrate solution was added to each cup and the electrode potential measured over the time period shown.

Transepidermal water loss (TEWL) was recorded with an electrolytic system (Meeco) using a published technique (Cunico *et al.*, 1977).

Effects of eccrine sweating

Water loss and chloride transport were measured on an anhidrotic individual before and after methacholine (0·1 cm^3, at 1 mg/ml). The anhidrotic subject had extensive burn scars on his forearms where the measurements were made. Water loss and chloride transport were similarly measured in a normal (control) individual.

Ambient conditions

To minimize sweating, studies were performed (except as noted) when the laboratory was below 21°C. Subjects were acclimatized to the laboratory for at least 30 min before testing.

Calculations

The raw data were in the form of mV per time increment: these were converted into molarity chloride per time increment using the standard curve (Fig. 1). A graph of molarity (mol dm^{-3}) chloride versus time was prepared for each experiment and the slope (determined using a linear regression programme) became the rate of chloride diffusion per minute. A graph of chloride molarity vs time is shown in Fig. 2. The rate was transformed into the more useful form of total moles chloride per minute per

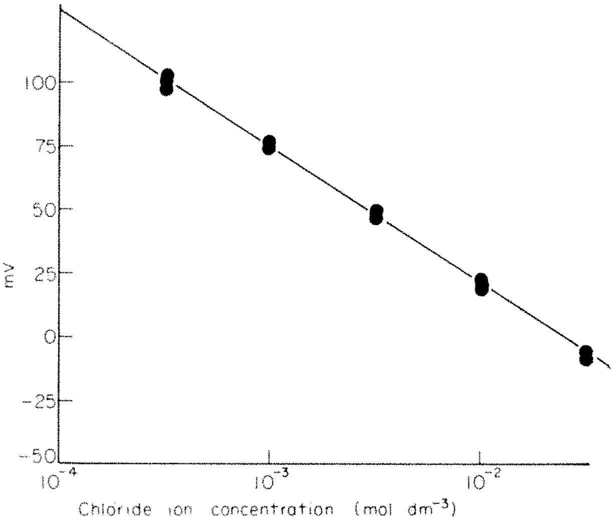

FIGURE 1. The increase of chloride concentration in the cup as a function of time. The slope is the rate of outward flux through the hydrated skin.

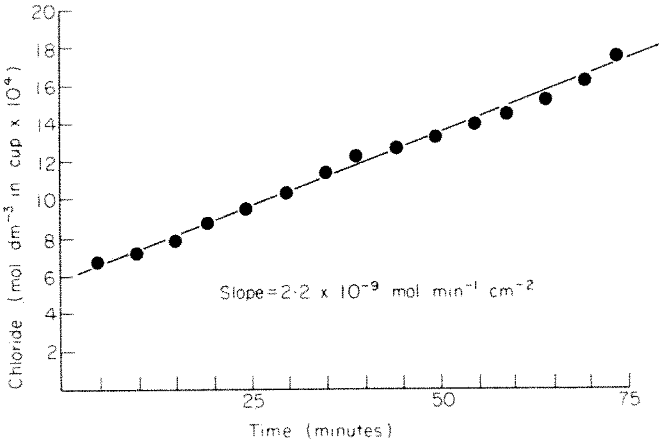

FIGURE 2. This calibration curve was used to assign chloride concentration from the specific ion meter's teadings in mV.

square centimeter, by multiplying the solution volume in litres and dividing by the cup's area. The results (Tables 1, 2 and 3) were determined by this method:

$$\text{Rate (mol cm}^{-3}\text{ min}^{-3}) = \frac{(\Delta\text{mol dm}^{-3})\,(\text{dm}^{3})}{(\Delta t)\,(\text{cm}^{-3})}$$

RESULTS AND DISCUSSION

An exponential decay of the chloride flux rate over time would be expected in theory; within the short time limit imposed upon the cup technique, the chloride concentration in the cup never approached

TABLE 1. Average chloride transport rates at various sites

Site	Rate cm^{-2}min^{-1} × 10^{9}	Subjects	*P*
Volar forearm	3·80 ± 2·63	14	—
Palm at 14^{0}	16·97 ± 3·15	4	0·0001
Palm at 24^{0}	30·4 ± 10·2	2	—
Calf	3·17 ± 1·41	4	0·63
Hand, dorsum	3·59 ± 4·01	3	0·87
Forearm, dorsum	2·02 ± 0·61	2	—

TABLE 2. Chloride transport rates after topical treatments

Treatment	Rate mol cm^{-2}min^{-1} × 10^{9}	Subjects	*P**
None	3·80 ± 2·64	14	—
DMSO	24·61 ± 32·18	5	0·02
Acetone	4·11 ± 2·35	4	0·83
Ethyl acetate	3·52 ± 3·26	3	0·87
10% SDS	4·66 ± 4·33	3	0·65
Cellophane tape stripping	64·76 ± 47·43	3	0·0001

*The *P* values are for unpaired Student's t comparison between the treated skin and the control values.

TABLE 3. Transepidermal water loss before and after methacholine stimulation in an anhidrotic individual

	TWL mg cm^{-2}h^{-1}	
Skin type	Before	After
Scar	119	118
Normal	100	Off scale

that of the underlying interstitial fluid at approximately 0·114 mol dm^{-3} (Fig. 2) (Maxwell & Kleeman, 1962). For each individual experiment the data were analysed using a standard linear regression program; in every case reported here the correlation coefficient was greater than 0·80. Therefore, the approximation of linearity of the chloride diffusion rate measured appears justified.

Wahlberg (1967) stated that electrolyte diffusion through the skin is principally epidermal with parallel paths via hair follicles, eccrine ducts, sebaceous ducts and apocrine ducts playing an inconsequential role. Experiments performed with pharmacological sweat inhibitiors (Pinson, 1942; Bettley

& Grice, 1965) have shown that active sweating occurs on the palms and plantar surfaces when the individual measured is resting at ambient temperature (20°C). Palmar skin has been shown to release sweat both at cool ambient temperatures and after the administration of pharmacological sweat inhibitor (Wolf and Maibach, 1974). As another test to determine if eccrine sweat duct activity had an effect upon the observed diffusion measurements, the following was done:

(1) Chloride flux (below) and water loss measurements (Table 3) were made on an individual with extensive anhidrotic burn scars.

(2) Similar measurements were performed on normal skin (Tables 2 and 3).

The methacholine stimulation in the anhidrotic individual had no effect upon chloride or on water loss, while it increased chloride measured an order of magnitude in a normal individual along with an off the scale increase in water emission (eccrine sweat). The anhidrotic site had a chloride flux (the forearm) of $6{\cdot}6 \times 10^{-9}$ mol cm^{-2} min^{-1}, within the range of normal chloride diffusion noted here. This suggests that the measurements performed at normal resting conditions are not significantly affected by sweating, and that the onset of active sweating is obvious and at a value one order of magnitude greater than normal non-sweating measurements.

The sites chosen for the anatomical variation of chloride flux were not significantly different except the palm. Palmar skin was significantly more permeable to chloride at room temperature 21°C and in an environmental chamber at 14°C (Table 1). Similar marked increase of TEWL of the palm compared to the forearm occurs (Burch & Windsor, 1946). The high palmar chloride flux and TEWL are most probably due to active sweating, both as a thermoregulator and to facilitate grip.

The chloride flux measurements after topical application of substances to the volar forearm are in Table 2. The only compound significantly changing the flux of chloride through the epidermis was DMSO. DMSO affects the stratum corneum barrier function and is also a solvent for electrolytes; it is not surprising that it affects chloride flux (Leake, 1967). DMSO has been shown to increase skin penetration of electrolytes (Kiss & Horvath, 1972) and water (Baker, 1968). The DMSO significantly increased the chloride penetration by one order of magnitude to $24{\cdot}6 \times 10^{-9}$ mol cm^{-2} min^{-1}. The alteration by the DMSO was before visible erythema and irritation were observed.

Cellophane tape stripping greatly increased chloride transport (Table 2), presumably by removing much (but not all) of the stratum corneum barrier to chloride loss.

The transport of chloride is facilitated by the presence of the aqueous reservoir attached to the skin. We suspect that the stratum corneum must be hydrated before chloride may be transported. In normal skin TEWL values range from 90 to 700 μg cm^{-2} h^{-1}, an amount of water vapour too dilute to be considered liquid. This TEWL is not likely to act as a major medium for transport of chloride in unhydrated skin; the static (chloride activities) measurements by Green, Mascia & Behrendt (1975) and Grice *et al.*, (1975) may be assumed to be due to dried residue of eccrine sweat and cellular debris of cornified cells.

We believe that the technique presented to determine the flux of chloride ions may become a useful method of assessing barrier function of the stratum corneum. The technique is sensitive enough to observe barrier damage caused by DMSO before it is visible and with no apparent change in TEWL observed. The technique is capable of differentiating between normal and palmar skin under conditions where no obvious eccrine sweating occurred. When a subject is sweating the chloride flux is of sufficient magnitude to be obvious. Interference by sweating is clearly discriminated by accurate data; the only apparent interference would be chloride salts of drugs or compounds applied to the skin.

The damage due to DMSO and lack of an effect with acetone, ethyl acetate and 10% SDS should be taken in the context of the acute single dosing; repetitive dosing or higher concentrations might yield a different result.

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Diffusion of oxygen through the mouse ear

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SUMMARY

Oxygen tension differences across the mouse ear have been measured polarographically under conditions of no blood flow. For some experiments the ear was split into two by cleavage along the central cartilage plate, and the diffusion of oxygen measured in both directions across these asymmetrical preparations. Measurements were also made on ears from which the stratum corneum had been removed by stripping with Sellotape. It was possible to relate these results to a simple multi-layer diffusion model. The main barrier to diffusion of oxygen resides in the stratum corneum, whose permeability is estimated to be $1{\cdot}2 \times 10^{-8}$ ml O_2 atm^{-1} cm^{-1} s^{-1}. The permeability of the rest of the ear is $4{\cdot}7 \times 10^{-7}$ ml O_2 atm^{-1} cm^{-1} s^{-1}.

The inhibition of tissue respiration by the local injection of solutions of sodium amytal, potassium cyanide and other substances reduced the oxygen gradients by factors of between 3 and 7. Cooling the ear from room temperature to 0°C reduced the gradients by a factor of about 4.

Oxygen is supplied to the skin both from the blood and by diffusion into the surface from the atmosphere. There will be gradients of oxygen tension dependent on local tissue oxygen consumption and the local blood perfusion rate. The gradients may thus fluctuate in time (Evans, Naylor & Sayers, 1977). Damage to cells caused by ionizing radiation is known to depend on the oxygen tension at the time of irradiation, but there is a lack of quantitative information on the factors determining oxygen tension at different levels in the skin.

As a first step towards irradiation of skin under controlled conditions of oxygenation, the oxygen tension gradients associated with the supply of oxygen from the atmosphere alone have been studied in conditions when there is no supply from the blood vessels. For this purpose the mouse ear was chosen as a suitable tissue since it is a thin double layer of skin with a blood supply which is simple to occlude. The ear consists of a thin (approximately 5 μm) layer of compact keratin forming the stratum corneum, about two layers of nucleated epidermal cells, and a dermis of more sparsely cellular connective tissue with a central plate of cartilage one cell layer thick. Hair follicles penetrate down almost to the cartilage plate. Small amounts of muscle and fat are present in some areas of the dermis. The total thickness of the ear is 150–250 μm.

In a homogeneous tissue, the oxygen gradients are determined by the oxygen permeability and by

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the tissue oxygen consumption rate. In a heterogeneous stratified tissue such as the mouse ear, there may be differences in both permeability and oxygen consumption rate in the layers. It will be shown here that changing the order of the layers can also profoundly alter the oxygen gradients and consequently the measured differences in oxygen tension across the full thickness of tissue. This enables information about the oxygen transport properties of the constituent layers to be obtained.

MATERIALS AND METHODS

The CFLP strain of mice was used in the majority of experiments. Other strains used were C57/BL, CBA/Ca, C3H/He and TO. The animals were used when they were 3–4 months old. They were anaesthetised by intraperitoneal injection of sodium pentobarbitone (Sagatal), and the hairs were removed from the ears by a three minute topical application of a proprietary cream containing potassium thioglycollate (Veeto). The cream and loose hairs were then washed off with water. For most of the experiments the animals were killed and the ears removed for the measurement of oxygen gradients. For some experiments, however, the measurements were carried out *in vivo* with the blood supply clamped off at the base of the ear with a device incorporating a small rubber tube (Paul's tubing) inflated to a pressure of 250 mm Hg on either side of the ear.

Those ears which were removed could be separated into two halves along the plane containing the cartilage plate by using a scalpel and gentle traction. The two halves could then be studied separately.

Attempts were made to modify the permeability of the keratin layer by soaking the ear before removal from the animal for 20 min in various preparations. Water, solutions of salicylic acid, urea, lauryl sulphate, lithium bromide, ammonium thiocyanate, and trypsin were used. Mechanical disruption of the keratin by abrasion, or removal by repeated stripping with sellotape were other methods used. In order to inhibit cellular oxygen consumption the ears were soaked in solutions of barbiturates. This procedure did not however change the oxygen gradients. The solutions were therefore injected at the base of the ear with a fine dental needle. 1% of hyaluronidase in the solutions facilitated the spreading of the injected material evenly throughout the ear. Solutions of deoxyglucose, sodium malonate, sodium azide, sodium fluoride, sodium fluoroacetate and potassium cyanide were also injected in this way in order to inhibit oxygen consumption. After injection the ear was compressed for 10 min by a lead weight to a pressure of about 250 mmHg before its removal from the mouse.

Oxygen tensions were measured polarographically using 25 μm diameter platinum cathodes and a silver/silver chloride anode with a polarizing voltage of 0·8 V. For the experiments on isolated tissue, one of two experimental arrangements was used.

In the first, which is similar to that used by Takahashi, Fatt & Goldstick (1966), the ear rests on the platinum cathode embedded in a 7 mm diameter glass rod (Fig. 1). Oxygen can diffuse into it at the upper surface, whereas no oxygen crosses the lower surface apart from the negligible amount consumed at the cathode surface. Either the full thickness of ear may be placed on the cathode, or one of the halves produced by splitting the ear and these may have either stratum corneum or dermis positioned uppermost.

In the second arrangement (Fig. 2) the ear is supported by a small frame so that oxygen is free to diffuse in from both surfaces. Oxygen tension is measured by inserting a sharpened glass-insulated platinum cathode obliquely into the ear so that its tip is adjacent to the cartilage plate. This is done manually under a dissecting microscope. A piece of lens tissue impregnated with saline forms the anode bridge.

In both arrangements the ear is enclosed in a gas-tight chamber which can be perfused by humidified gas with any desired oxygen content from a large syringe driven manually or automatically. The

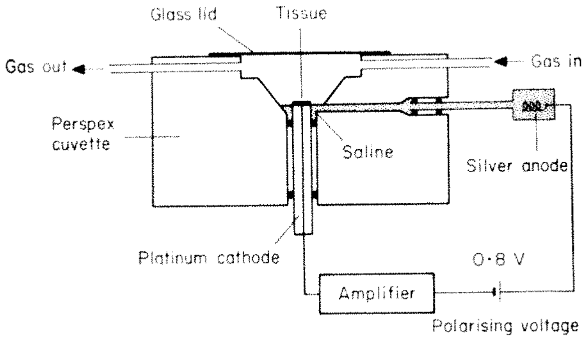

FIGURE 1. Apparatus used for measuring oxygen tension differences across the isolated mouse ear.

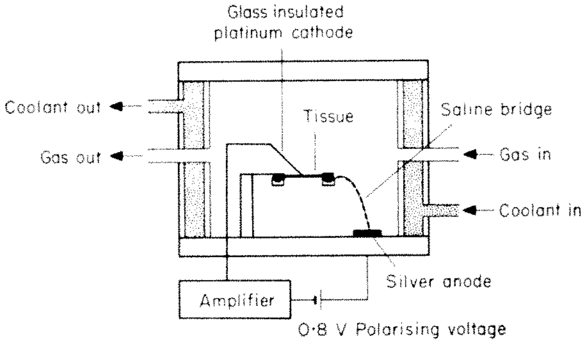

FIGURE 2. Apparatus used for measuring the oxygen tension at the centre of the mouse ear with a glass-insulated electrode.

effluent gas is discharged under a shallow water layer to prevent any back diffusion of gas when the perfusion stops. The chamber in Fig. 2 is surrounded by a hollow jacket through which a refrigerated liquid coolant (urea solution) may be circulated in order to measure the effect upon the oxygen gradients of cooling to temperatures down to 0°C.

To find the difference in oxygen tension between the surface of the ear and the cathode it is not necessary to measure absolute oxygen tensions, and therefore a membrane covering the electrode is not required. The rate of oxygen consumption by tissue is independent of oxygen tension down to very low tensions (Longmuir, Pistel & Magnus, 1958). Therefore there will be a linear relationship between the amount of oxygen in the gas in contact with the ear and the corresponding steady electrode current. By making measurements at several gas concentrations, a graph may be drawn (Fig. 3) of electrode current against oxygen tension in the gas phase. The oxygen tension corresponding to zero electrode current gives the oxygen tension difference across the ear preparation.

A small piece of polythene sheet 12·5 μm thick and of known permeability (Evans & Quinton, 1978) could be placed on the surface of the tissue in Fig. 1, and this increased the difference in oxygen tension between the gas and the tissue surface in contact with the cathode. The total oxygen consumption rate of the tissue can then be found, since the flux of oxygen through the membrane into the tissue can be calculated. Tissue thicknesses were measured visually by using a microscope with a small depth of focus.

In those experiments which were carried out *in vivo*, measurements were made with the ear lying

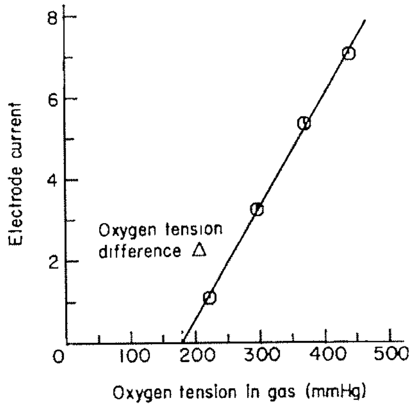

FIGURE 3. Relationship between electrode current and oxygen tension in the gas phase over the mouse ear. The oxygen tension difference across the tissue is the intercept on the oxygen tension axis.

on a glass-embedded cathode similar to that in Fig. 1, and with the blood supply to the ear occluded. Humidified gases of known oxygen content were blown over the upper surface through a tube at a rate sufficient to control the surface oxygen tension.

RESULTS

The oxygen tension differences Δ_1 to Δ_8 across ear tissue are shown in Table 1. Δ_1 to Δ_3 are the differences measured across the full thickness of ear (Δ_1), across one half of a split ear with the keratin side upwards and adjacent to the gas phase (Δ_2), and across a similar half of a split ear but with the

TABLE 1. Oxygen tension differences in CFLP mouse ear

Tissue preparation	Symbol	Oxygen tension difference (mm Hg) (±s.e. mean)
Full thickness ear	Δ_1	189·4±7·5
Half thickness ear with keratin next to gas	Δ_2	88·0±4·8
Half thickness ear with keratin next to cathode	Δ_3	14·8±1·6
Full thickness ear with keratin stripped from surface next to cathode	Δ_4	206·5±22·7
Full thickness ear with keratin stripped from surface next to gas	Δ_5	64·4±5·8
Half thickness ear with keratin stripped from surface next to gas	Δ_6	14·8±2·1
Half thickness ear with keratin stripped from surface next to cathode	Δ_7	12·6±1·9
Full thickness ear with electrode inserted to central plane	Δ_8	120·0±8·4

keratin downwards and in contact with the electrode (Δ_3). Removal of keratin from one side of the ear by repeated stripping with sellotape enables four more oxygen tension differences, Δ_4 to Δ_7, to be measured. These are for full thickness ear with the surface stripped of keratin in contact with the electrode (Δ_4), for the same but with the stripped surface in contact with the gas phase (Δ_5), for one half of a split ear with the stripped surface adjacent to the gas phase (Δ_6), and for the same but with the stripped surface in contact with the electrode (Δ_7). All these are for tissue placed on the glass-embedded cathode shown in Fig. 1. Δ_8 is the oxygen tension difference between the surface and centre of a full thickness ear measured with an electrode inserted into it (Fig. 2). The measurements were made at temperature (18–22 °C) and Table 1 shows the means and standard errors of Δ_1 to Δ_8. Groups of at least fifteen animals were used to estimate Δ_1 to Δ_7, and five animals for Δ_8. There was no significant difference in the values of Δ_2 whether the tissue included the central cartilage plate or not, and the value in the Table includes measurements of both kinds. Δ_8 is greater than Δ_2, and this arises from the difference in thickness of tissue used in the two cases. Electrode insertions, from which Δ_8 derives, were made at points in the ear which were about 2 mm further from the tip than those at which Δ_2 was estimated. In consequence of this the thickness of ear for the measurement of Δ_8 was about 30% greater, and this satisfactorily accounts for the larger value of Δ_8.

Measurements of the oxygen tension differences across the mouse ear *in vivo* with the blood supply occluded were consistent with the value of Δ_1, indicating that removal of the ear does not introduce any artefact into the measurements. A few measurements of Δ_1 on ears removed from mice killed without prior barbiturate anaesthesia also gave essentially similar values (174 ± 10 mm Hg).

Table 2 shows the oxygen tension difference Δ_1 across the complete thickness of the ear in CFLP mice compared with several other strains (four animals for each strain).

TABLE 2. Effect of mouse strain upon ear oxygen gradients

Mouse strain	Body weight (g)	Oxygen tension difference Δ_1 (mm Hg) (± s.e. mean)
CFLP	30–35	189·4 ± 7·5
TO	20–25	246·6 ± 20·2
C57/BL	18–22	308·6 ± 40·7
CBA/Ca	18–22	338·9 ± 31·1
C3H/He	18–22	338·9 ± 22·9

Mechanical abrasion of the stratum corneum by gentle rubbing with emery paper or by scratching with needles resulted in a reduction in the size of Δ_1 which on some occasions approached that produced by complete removal of the keratin layer by stripping with sellotape. It was notable that partial removal of the keratin layer by stripping had only a small effect on Δ_1.

A few measurements of Δ_1 on ears which had not been treated with Veeto on the surface in contact with the gas gave a value of 377 ± 38 mmHg, which suggests that the potassium thioglycolate reduces Δ_1 to about half of that in the normal ear.

None of the attempts to reduce Δ_1 further by soaking the ear in solutions which might alter keratin structure was successful, and neither was it found possible to introduce amytal into the ear by immersion in an aqueous solution, or by electrophoresis.

Table 3 shows the effect on Δ_1 of injecting solutions of several metabolic inhibitors into the ear before its removal. Each estimate of Δ_1 is based on a group of at least four animals. Cyanide, azide and fluoride reduced the value of Δ_1. Animals whose ears were injected with these, and then allowed to

TABLE 3. Effect of injecting metabolic inhibitors on the oxygen tension difference in CFLP mouse ears

Inhibitor	Strength (mmol/l)	Oxygen tension difference Δ_1 (mm Hg) (±s.e. mean)
None	—	189·4±7·5
Sodium amytal	25	25·0±3·6
	12	27·4±3·8
	6	38·3±5·5
	3	71·8±14·8
Potassium cyanide	25	34·8±1·6
	10	46·9±4·7
	1	166.8±24.4
Sodium azide	25	59·2±4·4
Sodium fluoride	25	98·4±15·5
Sodium fluoroacetate	25	133·2±22·9
Sodium malonate	25	213·9±48·8
Deoxyglucose	25	147·3±23·7

recover all showed slight ear damage 24 h later. Injection of sodium amytal, and to a lesser extent other barbiturates, also reduced Δ_1 considerably, and with these, little or no visible ear damage developed in animals which were allowed to recover. A concentration of sodium amytal as low as 12 mmol/l was effective in reducing Δ_1 down to about 14% of its normal value, and more concentrated solutions did not produce any further reduction.

The upper curve in Fig. 4 shows the relationship between temperature and the oxygen tension difference Δ_8 measured with an intradermal electrode in the ear. The lower curve shows the results of similar measurements made on ears which had been first injected with 25 mmol/l sodium amytal. The dependence on temperature in this case is less pronounced.

Placing a piece of 12·5 μm thick polythene sheet on the upper surface of the full thickness of ear in the apparatus of Fig. 1 increased the oxygen tension difference between the gas and the electrode by 107±11 mm Hg.

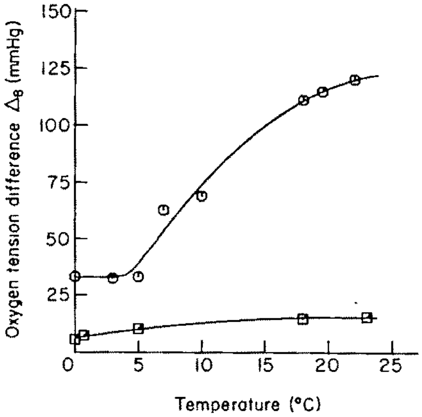

FIGURE 4. Relationship between the oxygen tension difference Δ_8 in the mouse ear and temperature for CFLP mice: upper curve—normal ear; lower curve—ear injected with 25 mmol/l sodium amytal.

DISCUSSION

The oxygen tension gradients

The histological structure of the ear suggests that a simple model consisting of five uniform layers may be a good approximation in considering the oxygen gradients within it. Two layers of stratum corneum overlie two flat sheets of epidermal cells between which lies the dermis. The presence or absence of the central cartilage plate within the dermis seems to have a negligible effect on the oxygen tension difference Δ_2, and the hair follicles have a spacing which is large compared with their depth, and they will also be neglected. We assume a stratum corneum of thickness x_1 and oxygen permeability k_1, an epidermal cell layer of thickness x_2, oxygen consumption rate per unit volume m_2 and permeability k_2, and a dermal layer of thickness x_3, oxygen consumption rate m_3, and permeability k_3. When the ear is split in two, there will be only three layers, the dermis now having a thickness $\frac{1}{2}x_3$. Examination of the skin after stripping with Sellotape suggests that virtually the whole of the stratum corneum has been removed. Assuming that the law of diffusion applies to oxygen transport through the ear, and that m_2 and m_3 are independent of oxygen tension, the oxygen tension differences Δ_1 to Δ_7 across the different combinations of constituent layers can be written down from the general equation for the oxygen tension difference Δ across n layers of tissue, the first being in contact with the gas phase, and the last in contact with an impermeable surface.

The equation is:

$$\Delta = \sum_{r=1}^{n} y_r\left(\tfrac{1}{2}\mu_r + \sum_{s=r+1}^{n} \mu_s\right)$$

where $\mu_r = m_r x_r$, $y_r = x_r/k_r$, and r refers to the rth layer of tissue. The derivation of this is given in the appendix. The equations for Δ_1 to Δ_7 can then be written:

$$\Delta_1 = \mu_2\,(2y_1+2y_2+y_3)+\mu_3(y_1+y_2+\tfrac{1}{2}y_3) \tag{1}$$
$$\Delta_2 = \mu_2\,(y_1+\tfrac{1}{2}y_2)+\mu_3(\tfrac{1}{2}y_1+\tfrac{1}{2}y_2+\tfrac{1}{8}y_3) \tag{2}$$
$$\Delta_3 = \mu_2(\tfrac{1}{2}y_2+\tfrac{1}{2}y_3)+\tfrac{1}{8}\mu_3 y_3 \tag{3}$$
$$\Delta_4 = \mu_2\,(2y_1+2y_2+y_3)+\mu_3(y_1+y_2+\tfrac{1}{2}y_3) \tag{4}$$
$$\Delta_5 = \mu_2\,(2y_2+y_3)+\mu_3(y_2+\tfrac{1}{2}y_3) \tag{5}$$
$$\Delta_6 = \tfrac{1}{2}\mu_2 y_2+\mu_3(\tfrac{1}{2}y_2+\tfrac{1}{8}y_3) \tag{6}$$
$$\Delta_7 = \mu_2(\tfrac{1}{2}y_2+\tfrac{1}{2}y_3)+\tfrac{1}{8}\mu_3 y_3 \tag{7}$$

From these equations it can be seen that there are four inter-relationships between these quantities, i.e.

$$\Delta_3 = \tfrac{1}{2}\Delta_1-\Delta_2 \tag{8}$$
$$\Delta_4 = \Delta_1 \tag{9}$$
$$\Delta_5 = \tfrac{1}{2}\Delta_3-\Delta_3 \tag{10}$$
$$\Delta_7 = \Delta_3 \tag{11}$$

The experimental values of Δ_1 to Δ_7 are of course subject to experimental error. If the experimental values given in Table 1 are slightly modified to the set:

$\Delta_1 = 200$, $\Delta_2 = 85$, $\Delta_3 = 15$, $\Delta_4 = 200$, $\Delta_5 = 63$, $\Delta_6 = 16{\cdot}5$, $\Delta_7 = 15$

(all measured in mm Hg) the relationships hold exactly, and this set of modified values is compatible with the experimental values with their associated errors. This provides support for the concept of a simple five-layer model.

Using the unmodified experimental values it is possible to estimate the properties of the different layers of tissue. It will be assumed that the permeability of the dermis, k_3, is the same as that of the epidermis, k_2. Although this assumption has no direct experimental basis, k_2 and k_3 are unlikely to differ widely in view of the composition of epidermis and dermis. Because of the four interrelationships, (8) to (11), there are only three independent oxygen tension differences, and Δ_1, Δ_2 and Δ_5 will be used. By solving the equations for these, the primary properties of the tissue layers can be written in terms of these oxygen differences as:

$$\frac{k_2}{k_1} = \frac{h}{x_1}\left(\frac{\Delta_1}{\Delta_5} - 1\right) \tag{12}$$

$$m_3 = \frac{k_2}{x_3 h}\left\{4\Delta_2 - 2\Delta_1 + \left(2 - \frac{x_2}{h}\right)\Delta_5\right\} \tag{13}$$

$$m_2 = \frac{k_2}{2x_2 h}\left\{2\Delta_1 - 4\Delta_2 - \left(1 - \frac{x_2}{h}\right)\Delta_5\right\} \tag{14}$$

where $h = x_2 + \frac{1}{2}x_3$.

From measurements on a number of ears, x_1, x_2 and x_3 were estimated to be about 5, 15 and 170 μm respectively at a distance of 2 mm from the tip of the ear, where Δ_1 to Δ_7 were measured. Hence from equation (12), we obtain $k_2/k_1 = 39 \pm 8$. This very high value of k_2/k_1 clearly shows the high barrier properties of the stratum corneum to oxygen, a situation which is paralleled by the barrier properties of this same layer to water vapour (Blank, 1953). Blank considered that the most significant part of the barrier is situated at the base of the stratum corneum, a finding which is in accord with our findings that the oxygen tension difference across the ear is significantly reduced by Sellotape stripping only when this is carried virtually to completion.

In considering the supply of oxygen into both sides of the ear from the atmosphere, it is interesting to know what proportion of the fall in oxygen tension from the surface to the middle of the ear occurs over the stratum corneum. This fraction F is given by:

$$F = \frac{1}{2}\frac{k_2 x_1 \Delta_5}{k_1 h\ \Delta_2} \tag{15}$$

Using the values for Δ_2, Δ_5 as before, F is 0·7, i.e. 70% of the fall in oxygen tension occurs across the stratum corneum.

It was remarkable that all attempts to increase the oxygen permeability of the thioglycollate treated stratum corneum by further chemical treatment were unsuccessful. The absolute permeability k_1 of the stratum corneum can be found if the permeability of the underlying epidermis and dermis, k_2, can be measured. The measurement of the increase in oxygen tension difference across the ear, which results from a layer of polythene being placed on the surface of the ear, enables this to be done. If this increase in oxygen tension difference is Δ_g, then:

$$k_2 = \frac{h\Delta_g k_m}{x_m \Delta_5} \tag{16}$$

where x_m, k_m are the thickness and oxygen permeability of the polythene layer, and k_m is $0{\cdot}35 \times 10^{-7}$ ml O_2 atm^{-1} cm^{-1} s^{-1} (Evans & Quinton, 1978). If Δ_g is 107 mm Hg, k_2 is $4{\cdot}7 \times 10^{-7}$ ml O_2 atm^{-1} cm^{-1} s^{-1}, which is about 75% of the permeability in water. The permeability of the hydrated stratum corneum k_1 is therefore $1{\cdot}2 \times 10^{-8}$ ml O_2 atm^{-1} cm^{-1} s^{-1}, although the value near the base of this layer could be even lower if the main barrier resides there. It should be noted that this estimate of k_1 is for skin which has been treated with potassium thioglycollate, and that this has a chemical effect on keratin. The value of Δ_1 for untreated skin, 377 mm Hg, is larger by a factor of about 2, so that k_1 for the untreated stratum corneum may be as low as about $0{\cdot}5 \times 10^{-8}$ ml o_2 atm^{-1} cm^{-1} s^{-1}.

An investigation of the sensitivity of k_2/k_1, m_2 and m_3 to errors in the experimental values Δ_1, Δ_2 and Δ_5 shows that, whereas k_2/k_1 is fairly insensitive to these, m_3 and particularly m_2 are both quite sensitive. However the mean oxygen consumption rate $\overline{m}$ for the whole ear is less sensitive to experimental error, and is given by the expression:

$$\overline{m} = \frac{2m_2\,x_2 + m_3\,x_3}{2h} \tag{17}$$

By using equations (13), (14) and (16), this can be written:

$$\overline{m} = \frac{k_m\,\Delta_g}{2h\,x_m} \tag{18}$$

From the experimental data, $\overline{m}$ is calculated to be 0·7 μl o_2 per mg wet weight per h at room temperature. This may be compared with the value of 2·56 μl o_2 per mg wet weight per h at 37°C obtained by Cruickshank & Cooper (1955) for slices of mouse ear skin suspended in Krebs-Ringer phosphate medium. Taking a factor of 2·6 per 10°C for the temperature dependence of the oxygen consumption rate, their value becomes 0·6 μl o_2 per mg wet weight per h at room temperature. This is in good agreement with the present estimate, and shows that there is no lack of oxidisable substrates under our conditions of measurement.

In those experiments which were performed *in vivo* with an occluded blood supply, it was found that the oxygen tension difference remained fairly constant for some hours after occlusion and was essentially the same as that in the isolated ear. Sufficient metabolites must therefore remain available for respiration in the cells to enable them to continue utilising oxygen at a constant rate for this period. If the animals were allowed to recover, no gross damage developed in the ear even though occlusion had been for four hours or more. The fall in tension through the living epidermal cells and dermis together is about 15 mm Hg at room temperature. Cooling to below 5°C or injection of sodium amytal would reduce this oxygen gradient to about 5 and 2 mm Hg respectively. Since one purpose of this work was to find a means of investigating the response of the living cells of the ear to ionising radiation at different concentrations of oxygen in a quantitative way, either of these procedures may provide the necessary conditions of a very low oxygen tension gradient.

It is worth emphasising that, in the experiments on half thickness ear with the method illustrated in Fig. 1, there is a considerable difference between the oxygen tension differences Δ_2 and Δ_3 depending on whether the dermis or the stratum corneum is in contact with the gas phase. This asymmetry in the two directions of oxygen diffusion arises from the differences in permeability and oxygen consumption rate in the component tissue layers, together with the differences in oxygen flux at the two surfaces. The boundary conditions are such that the whole of the oxygen supply to the respiring tissue layers is supplied from the gas but there is zero flux of oxygen into the surface adjacent to the electrode. Fig. 5 illustrates the situation. In curve (a), the stratum corneum is in contact with the air, and there is a steep fall in oxygen tension through this rather impermeable layer arising from the oxygen demand of the epidermis and dermis. For curve (b), the more permeable dermis is in contact with the air, and there is only a small oxygen tension gradient in it. In this instance there is no fall in tension across the stratum corneum as no oxygen is used in or beyond it. The first case is similar to atmospheric oxygen penetrating ischaemic skin, whereas the second resembles the situation existing when surface oxygen electrodes are used for transcutaneous monitoring of blood oxygen tension *in vivo*.

The ability to distinguish the different permeability properties of the layers of multi-layer structured

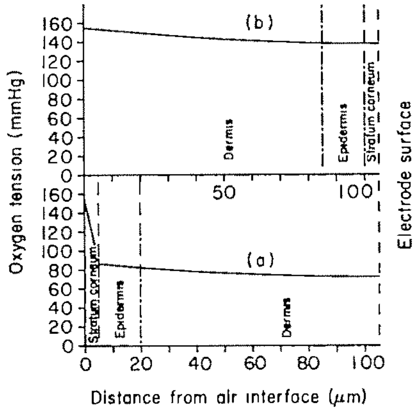

FIGURE 5. The asymmetry of gradients of oxygen tension across half thickness mouse ear: (a) stratum corneum in contact with air; (b) dermis in contact with air.

tissue without the need to separate them physically may prove of use in investigating more complex systems in which there are changes in the properties of one or more of the layers. The demonstration of the low permeability of the thin stratum corneum of the mouse ear is one such result of the application of simple diffusion theory to a multi-layer system.

Changes in oxygen consumption

The failure to introduce barbiturates into the ear by external application or by electrophoresis was consistent with earlier findings on human skin (Naylor & Evans, 1970). It appears to be necessary to remove the whole of the stratum corneum before penetration can occur either in human or murine skin.

The reduced oxygen tension differences across the ear after intradermal injections of sodium amytal solution are caused by the reversible inhibition of oxygen consumption in the cells (Chance & Hollunger, 1963). The intradermal injections appear to have no more damaging effect on the ears than the production of a transient erythema, which disappears within 24 h. The oxygen tension difference Δ_1 was also normal after 24 h. The inhibition appears to be maximal with an injected concentration of 12 mmol/l sodium amytal, but it is not complete. About 14% of the oxygen utilisation appears to persist even at amytal concentrations as high as 50 mmol/l suggesting that other pathways of metabolism are present in skin apart from those investigated by Chance & Hollunger (1963).

Oxygen consumption rate normally falls by a factor of about 2·5–2·8 per 10°C fall in temperature, which causes the oxygen tension difference Δ_8 to be reduced at lower temperatures. However this effect will be complicated by other changes, including changes in tissue permeability. This may account for the non-exponential dependence of Δ_8 upon temperature. A reduction of Δ_8 by a factor of about 4 is found for a 20°C temperature fall. After the injection of sodium amytal, the dependence of Δ_8 upon temperature was less, amounting to a factor of only 2·0 for a 20°C temperature fall. This again suggests that the residial oxygen consumption after amytal inhibition involves some different pathway.

The oxygen tension differences Δ_1 for different mouse strains given in Table 2 are of interest in that there is an inverse correlation of Δ_1 with body weight. Also the CFLP and TO strains, which are albino, have smaller values of Δ_1 than the other three strains, which are all pigmented. It is possible that these relationships are attributable to the higher metabolic oxygen requirements of the smaller pigmented strains, but the possible effect of differences in thickness or permeability of the stratum corneum cannot be excluded.

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APPENDIX

The oxygen tension difference across n layers of metabolising tissue

We assume (Fig. 6) n layers of tissue which consume oxygen, the rth layer having thickness x_r, oxygen consumption rate m_r and permeability k_r. The oxygen tension on the right hand side of the rth layer is p_r and that on the left hand side of the first layer is p_o, the fixed po_2 of the gas in contact with the tissue. There is no flux through the right hand boundary of the nth layer which is in contact with the electrode (or is a plane of symmetry).

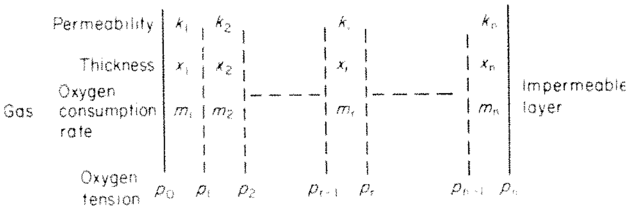

FIGURE 6. Model for diffusion in n layers of tissue. The left-hand boundary is in contact with gas at oxygen tension p_0, and the right hand boundary at tension p_n is impermeable to oxygen.

The diffusion equation for the rth layer is:

$$k_r\frac{\delta^2 p}{\delta x_2} = m_r \qquad (1)$$

The solution is:

$$p = \frac{m_r x^2}{2k_r} - Ax + p_{r-1} \qquad (2)$$

x being distance measured from the left hand boundary of this layer, The fall in po_2 across this layer is Δp_r ($= p_{r-1} - p_r$) and is therefore:

$$\Delta p_r = Ax_r - \frac{mx^2r}{2k_r} \tag{3}$$

The flux of oxygen out of the right hand boundary, f_r, is given by:

$$f_r = -k_r \frac{\delta p}{\delta x}\Big|_{x_r} = Ak_r - m_r x_r \tag{4}$$

Eliminating A, the constant of integration, from equations (3) and (4):

$$\Delta p_r = y_r(\tfrac{1}{2}\mu_r + f_r) \tag{5}$$

where $y_r = x_r/k_r$ and $\mu_r = m_r x_r$.
Since the flux f_n out of the right hand side of the tissue is zero because of the boundary conditions, f_r is simply the sum of the total oxygen consumption rate in the tissues to the right of the rth layer, that is:

$$f_r = \sum_{s=r+1}^{n} \mu_s \tag{6}$$

The oxygen tension difference Δ across all n layers is therefore:

$$\Delta = \sum_{r=1}^{n} \Delta p_r = \sum_{r=1}^{n} y_r(\tfrac{1}{2}\mu_r + \sum_{s=r+1}^{n} \mu_s) \tag{7}$$

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Age-related changes in human dermal elastic fibres

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SUMMARY

Age-related changes in the human dermal elastic fibres were studied by scanning (SEM) and transmission (TEM) electron microscopy. The SEM findings showed an increase in the complexity of shape and arrangement of the fibres including flattening and branching, an increase in the roughness of the surface, and a decrease in interfibrillar areas. The TEM findings showed a decrease in microfibrils and amorphous material, and an increase in electron dense inclusions followed by the appearance of vesicular structure.

There have been several reports on the age-related changes in the dermal elastic fibres as shown by light microscopy (Hill & Montgomery, 1940; Dick, 1947; Ma & Cowdry, 1950; Marshall, 1965; Mitchell, 1967; Montagna, 1973; Jarrett, 1974). However, there are variations in these reports. Some (Dick, 1947; Montagna, 1973; Jarrett, 1974) described thickening of the elastic fibres, others (Ma & Cowdry, 1950; Mitchell, 1967) described a decrease in amount and thinning of the fibres, and yet others (Hill & Montgomery, 1940; Marshall, 1965) found no significant changes.

On the other hand, there are only a few reports on the age-related changes in the elastic fibres as shown by transmission electron microscopy (Danielsen & Kobayashi, 1972; Stadler & Orfanos, 1978), and the results have been almost the same as follows:

(a) decreasing number of microfilaments, (b) the appearance of electron dense inclusions or a mass of dense grains in the elastin matrix, and (c) fragmentation and disintegration of the fibres, or the appearance of round holes.

In addition to the light microscopic (LM) and transmission electron microscopic (TEM) studies, scanning electron microscopic (SEM) studies might give us new information about the three-dimensional architecture of the dermal elastic fibres, although there is no previous report on this subject.

The purpose of this paper is to demonstrate the SEM findings as well as the TEM findings of the age-related changes in the dermal elastic fibres.

MATERIALS AND METHODS

Specimens were taken from the buttock of three groups of healthy persons: children (11 months and

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6 years of age), young adults (20 and 25 years of age) and old adults (65 and 80 years of age). These specimens were processed for SEM and TEM.

For SEM, a new technique was used (Tsuji, Lavker & Kligman, 1979). The specimens were incubated in 2 N sodium bromide at 37°C for 30 min, after which the epidermis was gently separated from the dermis. Approximately 1 mm thick transverse sections of the dermis were autoclaved in water for 8 h at 121°C at 18 lbs/in^2. The autoclaved specimens were fixed at room temperature in 2% paraformaldehyde-2·5% glutaraldehyde in 0·1 M cacodylate-HCl, buffered at pH 7·4 (Karnovsky, 1965) for 1 h, and then rinsed in buffer for 1 h after dehydration in a graded (70–100%) series of ethanol baths. The samples were then placed in 100% xylol for 2 h and air dried. Dried samples were mounted on aluminium stubs using silver paint. They were coated with a thin layer of gold palladium in an ISI sputter coater and viewed in a TV Mini-SEM operated at 10 kV.

For TEM, the specimens were fixed at 4°C for 1 h in 2% glutaraldehyde buffered with 0·2 M phosphate (pH 7·4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0·2 M phosphate (pH 7·4) for 1 h and then dehydrated by graded ethanol concentrations and embedded in Epon 812. Ultrathin sections were cut with a Porter-Blum MT-2 ultramicrotome. Some sections were stained with uranyl-acetate solution for 1 h and with Reynold's lead citrate for 10 min. Others were stained with tannic acid-uranyl acetate solution* (Kajikawa *et al.*, 1975) for 10 min and with Reynold's lead citrate for 10 min. They were examined with a Hitachi HS9 electron microscope.

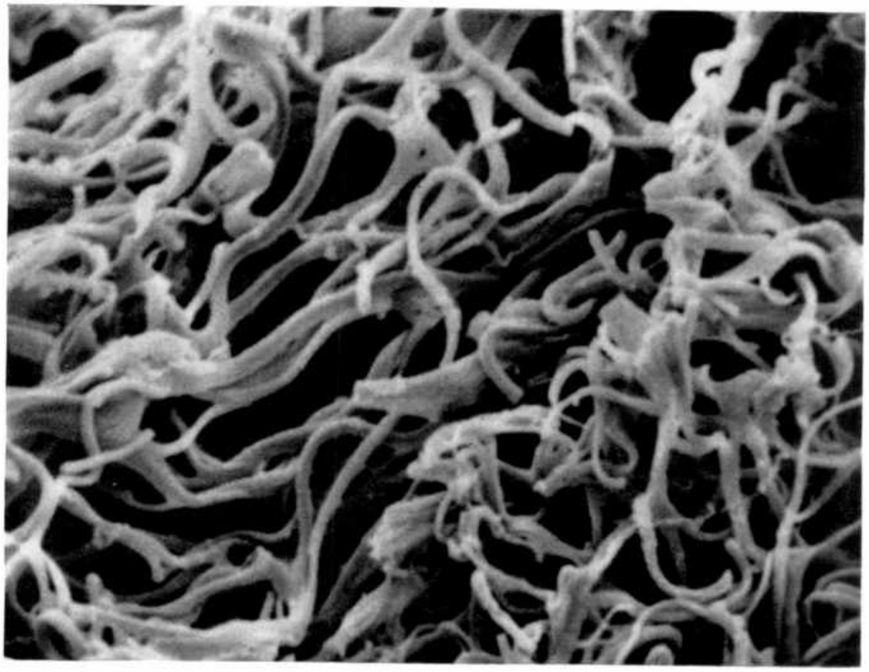

FIGURE 1. SEM findings of dermal elastic fibres at 20 years of age. Ribbon-like elastic fibres are aligned primarily in the same direction. They are cylindrical or elliptical and have a smooth surface (×910).

*The tannic acid-uranyl acetate solution was prepared as follows: 0·2 g of tannic acid and 0·5 g of paranitrophenol were dissolved completely in 35 ml of distilled water with the aid of heat. After cooling, 0·2–0.4 ml of 5% uranyl acetate was added to this solution.

RESULTS

SEM findings

The elastic fibres in young adult skin appeared slender, smooth and had relatively regular shape and arrangement (Fig. 1). In the papillary dermis, fine elastic fibres were aranged in a vertical network; they were predominantly cylindrical (0·5–2·5 μm in diameter). In the mid- and the deep dermis, ribbon-like elastic fibres were aligned, mainly horizontally, to the skin surface (Fig. 1). These were more elliptical and larger (1.5–3.0 μm in diameter), and showed more branching than the superficial fibres. Wide spaces were present among the elastic fibres throughout the dermis.

In old skin the elastic fibres appeared thicker and more irregular in shape than those of young subjects, and had a rough surface (Fig. 2). Vertically arranged fine fibres were not seen, in distinction to the papillary dermis of the young adults. In the upper dermis they appeared elliptical rather than cylindrical (2·0–3·5 μm in diameter) and were aligned primarily horizontally to the surface. In the mid- and the deep dermis, the fibres ran in various directions, forming complicated networks, and were larger, more elliptical, and more branched than the superficial fibres (Fig. 2). In the deep dermis they were often organized into large, thin sheets (up to 1 × 35 μm) with the surface of fine grooves aligned parallel to the long axis of the fibres (Fig. 2). There was much less space between the fibres than in the younger dermis.

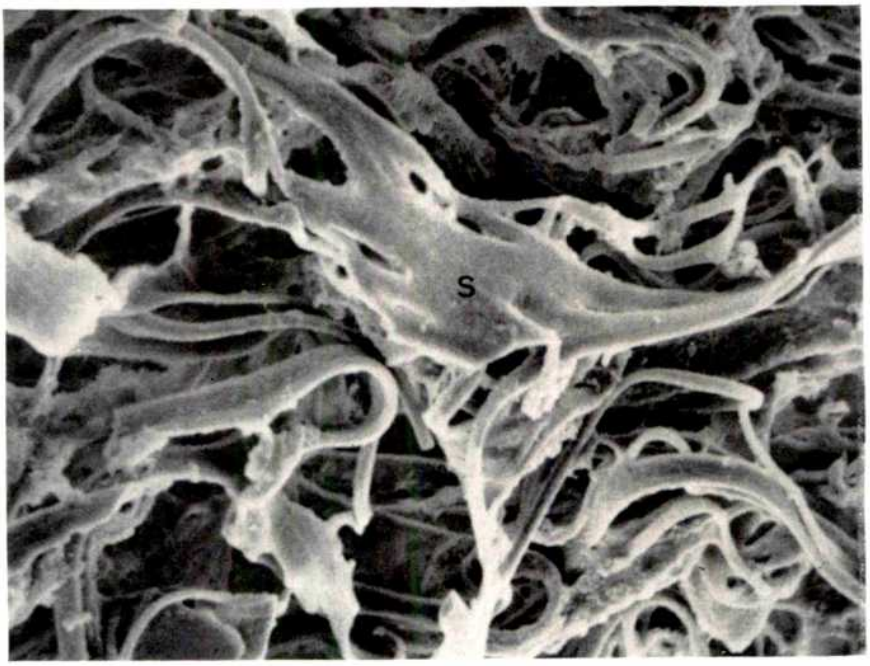

FIGURE 2. SEM findings of dermal elastic fibres at 62 years of age. The elastic fibres run in various directions, forming complicated networks. These are larger, more elliptical, and more branched than those in young adults' skin. A sheet-like fibre (S) with a rough surface is seen in the centre of this picture (×910).

TEM findings

Conventional staining (*uranium-lead staining*). Elastic fibres in the children's skin were slender and straight (Fig. 3). In the centre of these fibres there was an electron lucent area named 'amorphous material'. Many microfibrils were seen at the periphery, running parallel to the long axis of the fibres; some microfibrils were also present within the amorphous material.

Elastic fibres in young adults' skin were larger than those of the children's skin, and their margin was somewhat irregular (Fig. 4). The central part of the fibres was occupied by amorphous material, and the microfibrils were thicker than those in children's skin. These were located mainly at the periphery of the fibre, but some were also within the amorphous material.

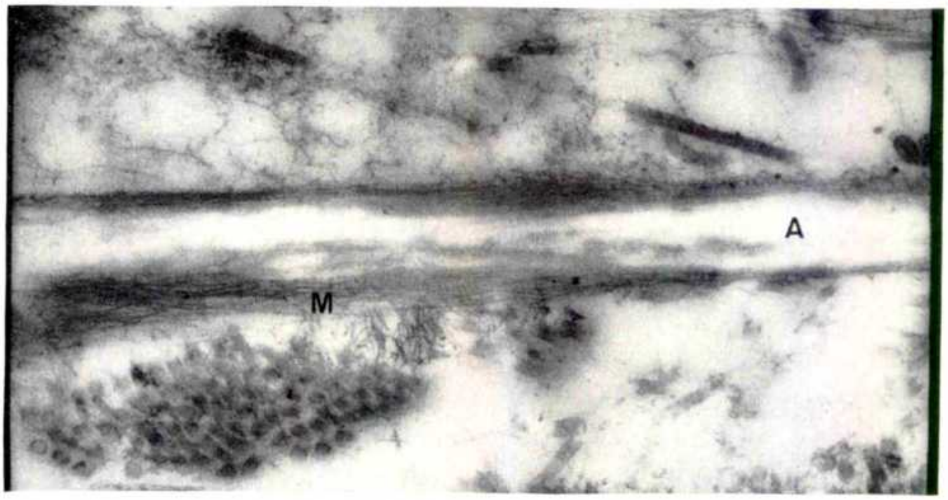

FIGURE 3. TEM findings of a dermal elastic fibre at 11 months of age. The elastic fibres are slender and straight. Amorphous material (A) is seen in the centre of the fibre. Many microfibrils (M) are present at the periphery, running parallel to the long axis of the elastic fibre. Some microfibrils are also present within the amorphous material (uranium-lead staining, × 31,500).

Elastic fibres in old skin were larger and more rough than those in young adult's skin. There were fewer areas of amorphous material and many fewer microfibrils than in the young adult's skin. Irregular and variously sized electron-dense inclusions were seen within the amorphous material. These inclusions often contained vesicular structures of about 200–350 nm in diameter.

Tannic acid staining. In general, amorphous material showed marked affinity for tannic acid and stained black, while microfibrils, as well as the electron-dense areas seen in conventionally stained sections, showed no affinity and stained relatively lightly.

In children's skin the central part of the elastic fibres stained homogeneously and intensely black. A large number of microfibrils were seen moderately electron-dense at the periphery of the elastic fibres.

In young adults' skin relatively large parts of the centre of the elastic fibres stained black but not homogeneously as seen in the children's skin. Moderately electron-dense areas consisting of fine granules and microfibrils were seen at the periphery and in the centre of the elastic fibres (Fig. 5).

In old skin small and irregularly dispersed areas in the centre of the elastic fibres stained black. Moderately electron-dense areas, consisting mainly of the fine granules, occupied large parts of the elastic fibres.

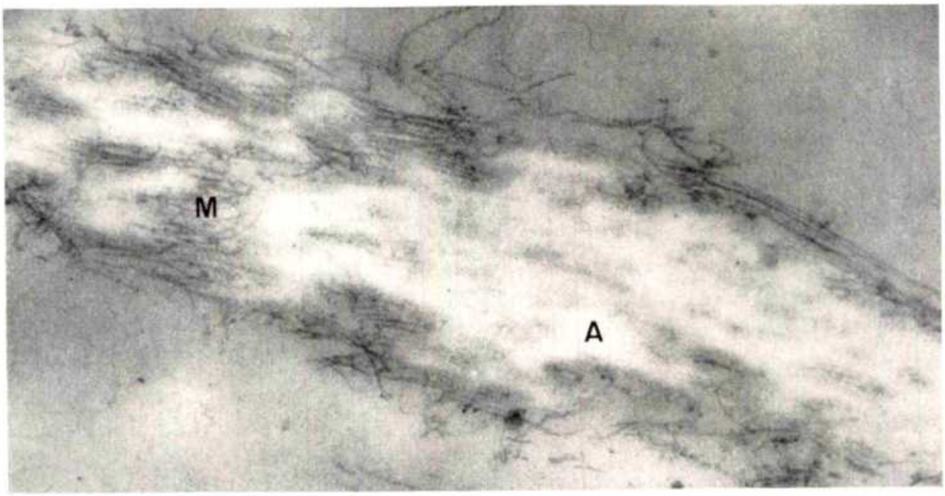

FIGURE 4. TEM findings of a dermal elastic fibre at 20 years of age. The elastic fibre is much larger than that at 11 months of age, and its margin looks somewhat irregular. A large central part of the fibre is occupied by the amorphous material (A). Microfibrils (M) are seen at the periphery as well as in the centre of the fibre (uranium-lead staining, × 31,500).

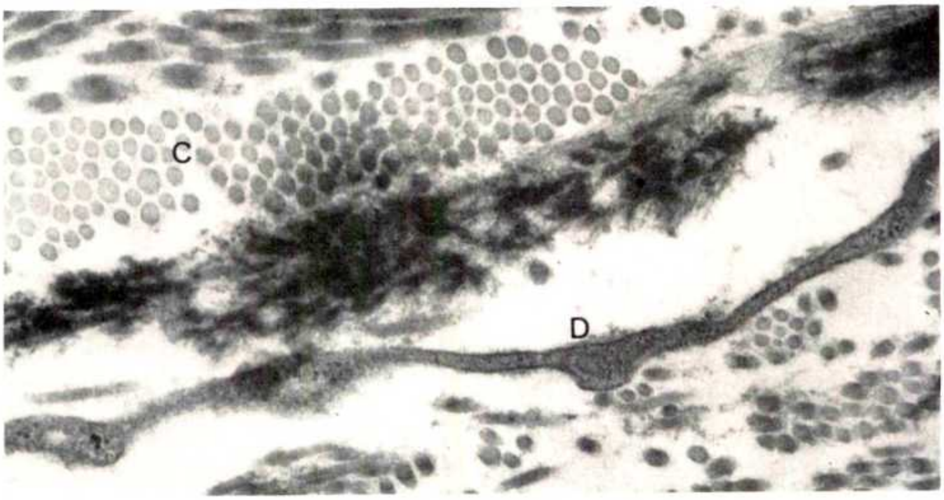

FIGURE 5. TEM findings of a dermal elastic fibre at 20 years of age. Relatively large parts of the centre of the elastic fibre stain black but not homogeneously. Moderately electron-dense areas consisting of fine granules and microfibrils are seen at the periphery and in the centre of the elastic fibre: C—collagen fibres; D—part of a fibroblast (tannic acid-uranium-lead staining, × 21,000).

DISCUSSION

The SEM findings in the present study revealed that there are differences in the three-dimensional architecture of the elastic fibres between the young and old individuals. The difference in the distribution pattern of the elastic fibres between the two age groups is similar to that shown in light microscopic studies by some investigators (Montagna, 1973; Jarrett, 1974). Other factors such as shape, arrangement, number of branches and surface appearance were also noted in this study.

There have been disagreements concerning the difference in thickness of the elastic fibres between young and old skin (Hill & Montgomery, 1940; Dick, 1947; Ma & Cowdry, 1950; Marshall, 1965; Mitchell, 1967; Montagna, 1973; Jarrett, 1974) in light microscopic studies. This is reasonable since most elastic fibres of old skin are elliptical and appeared either thicker, thinner or unchanged when compared with those of the young skin, depending on how the fibres were cut. Also differences in fixation techniques can readily alter the optical and electron microscopic appearances of the fibres.

The reason why the elastic fibres increase in thickness and amount with ageing is not clear. There are two different concepts to explain this (Jarrett, 1974). One is that the turnover rate decreases with ageing and the slower replacement of old fibres results in their being increasingly cross-linked. This consequently increases the relative quantity of fibrous protein in the dermis. The other is that mature elastin once formed remains in a static inert state throughout life. This postulate implies that the changes occurring with age are an increased aggregation of the already existing fibres together with a concomitant oxidation of protein-polysaccharide complexes.

The differences in ultrastructure of the elastic fibres with increasing age are also characteristic. Mature elastic fibres consist of two components: the central amorphous material and the peripheral microfibrillar components (Greenlee, Ross & Hartmann, 1966; Ross & Bornstein, 1969). In general, the microfibrils are thought to be the first structures to appear in elastogenesis in the fetus. Later in development, the conventional morphology of elastic tissue begins to manifest itself in the form of amorphous loci embedded within the fibrillar mass. As maturation of elastic tissue progresses, the amount of amorphous material increases and microfibrils decrease (Fahrenbach, Sanberg & Cleary, 1966; Greenlee *et al.*, 1966).

The elastic fibres at 11 months of age appear mature since they contain large central regions of amorphous material surrounded by microfibrils (Fig. 3). However, in comparison with those of older persons, the fibres and the microfibrils are much thinner and straighter. Many microfibrils are present within the amorphous material. The elastic fibres at 6 years of age, however, show almost the same features as those of the young adults, and this suggests that maturation of elastic tissue continues several years after birth.

As for the elastic fibres of old adults, our findings are similar to those demonstrated in some previous reports (Danielsen & Kobayashi, 1972; Stadler & Orfanos, 1978). According to Stadler & Orfanos (1978), structural alterations of ageing of dermal elastic fibres in unexposed skin begin between the ages of 30 and 50 years. These are a decrease in microfibrils, an increase in electron-dense inclusions followed by the appearance of vesicular structures, and the fragmentation and disintegration of the elastic fibres. They also stated that ageing of the fibre includes diminished synthesis of structural glycoprotein (microfilaments) and increasing content of osmiophilic groups, such as polar amino acids, fatty acids and calcium salts.

The findings with elastic fibres stained with the tannic acid-uranyl acetate combined technique showed quite different appearances from those of uranyl acetate alone. This underlines the important fact that different techniques give different light and electron microscopic appearances to dermal structures. Comparison of Figs 4 and 5 clearly demonstrates this point with the dermal elastic fibres from a 20-year-old subject. Tannic acid has been used in the leather industry for years and it is well known that it alters dermal components during the 'tanning process'. These differences are possibly related to the attachment of tannic acid complexes with uranyl acetate to chemical moieties of the elastic fibres.

Although fixation and staining procedures are essential for many techniques used for light and electron microscopic examination of tissues, it is only by the examination of relatively untreated materials that one will finally understand the physical state of the dermis in the living skin.

With the tannic acid method the central amorphous material is homogeneously present throughout the central region of the elastic fibres in young skin. This contrasts with the irregularly scattered amorphous material seen as fine granular and electron-dense inclusions in the conventionally stained sections. The increase in elastase-resistance of elastic fibres in old skin (Bodley & Wood, 1972; Jarrett, 1974) might be related to the presence of these inclusions.

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Bullous pemphigoid: the frequency of mucosal involvement and concurrent malignancy related to indirect immunofluorescence findings

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SUMMARY

A review of 124 cases of bullous pemphigoid was made and the clinical findings correlated with the results of indirect immunofluorescence. A circulating IgG basement membrane zone (BMZ) antibody was detected in the serum of eighty-nine (72%) of the patients. Antibody titres in these patients did not correlate with the extent of the disease or with the presence of mucous membrane lesions. Indeed, oral lesions were more common in the thirty-five sero-negative patients with 17% of such patients being affected compared to only 10% of those with positive indirect immunofluorescence findings. This difference, however, was not statistically significant.

Concurrent malignant disease (i.e. malignancy occurring within 6 months of pemphigoid being diagnosed) occurred in eight of the seronegative group (23%) and in only four of the eighty-nine seropositive cases (4%); this difference was significant. The association of bullous pemphigoid and malignant disease still remains controversial, but those who believe that the relationship is coincidental must now explain why concurrent malignant disease is more common in patients who have bullous pemphigoid and negative indirect immunofluorescence findings.

The clinical and immunopathological findings in bullous pemphigoid are now well established (Lever, 1953; Jordon *et al.*, 1967; Person & Rogers, 1977; Ahmed, Maize & Provost, 1977b). Despite this, doubts still persist concerning the frequency of mucosal involvement and the possible association of bullous pemphigoid with other diseases, particularly malignant neoplasms. Recently, it has been suggested (Person & Rogers, 1977) that concurrent malignant disease and mucosal involvement occur more commonly in those patients with bullous pemphigoid who have negative indirect immunofluorescence (IMF) findings.

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This survey of 124 cases of bullous pemphigoid considers the frequency of mucosal involvement and the possible association of concurrent malignancy with special reference to the presence or absence of demonstrable circulating IgG anti-basement membrane zone (anti-BMZ) antibodies.

MATERIALS AND METHODS

Sera were examined from 124 cases of bullous pemphigoid referred for diagnostic evaluation to the immunopathology section of the Department of Experimental Pathology, Institute of Dermatology, London. The diagnosis of bullous pemphigoid was established clinically by the referring physician and supported by compatible histopathological findings in all but four patients who did not have a skin biopsy. However, in these four cases a circulating anti-BMZ antibody was detected.

Clinical information was supplied by the referring physician on a questionnaire, often after a retrospective review of the case notes. The disease was graded as extensive or limited, i.e. confined to one region of the body or manifested by a small number of lesions in more than one region. Follow-up from the time of onset of symptoms varied from 6 months to 6 years. Malignant disease was defined as concurrent if it occurred within 6 months of the diagnosis of pemphigoid being established.

Immunofluorescence techniques for the demonstration of anti-BMZ antibodies followed standard procedures and have been described elsewhere (Beutner *et al.*, 1973; Hodge *et al.*, 1978). Briefly, serum was tested for the presence of an anti-BMZ antibody in dilutions of not less than 1:10 using commercially obtained anti-IgG conjugate against human skin substrate.

RESULTS

Clinical features

The results are considered in two sub-groups—'seropositive' (i.e. anti-BMZ antibody present at a titre of 1:10 or greater) and 'seronegative'

'*Seropositive cases*'. This group consisted of eighty-nine cases (72% of the total), forty-four female and forty-five male, aged 39–98 years. Fifteen had limited cutaneous involvement, predominantly affecting the lower limbs, whilst in the remainder the disease was extensive (Table 1).

Mucosal lesions were present in nine patients (10% of this sub-group), seven of whom had extensive cutaneous involvement. None of these had a concurrent malignant disease and the mucosal lesions cleared promptly with therapy in five. Antibody titres did not seem to correlate with the extent of the disease or with mucosal involvement. Concurrent malignant disease was present in four cases (4%), aged 62–85 years (mean 73 years); three having relatively high anti-BMZ antibody titres (Table 1). All four had extensive blistering without mucosal involvement. Three were alive 12 months after the discovery of their neoplasms whereas the fourth died from carcinoma of the bladder 8 months after diagnosis.

'*Seronegative cases*'. This group consisted of thirty-five patients, twelve female and twenty-three male, aged 22–87 years. Five showed limited cutaneous involvement whilst in the remaining thirty it was extensive.

Mucosal lesions were present in six patients (17% of this sub-group), all of whom had extensive skin disease and two of whom had concurrent malignant neoplastic disease.

Concurrent malignant neoplastic disease was diagnosed in eight patients (23% in this sub-group, mean age 62 years) (Table 1). Six had widespread bullae, and in two there were mucosal lesions. Five patients died from their malignancy within 9 months.

TABLE I. Bullous pemphigoid: clinical features related to initial indirect immunofluorescence findings

	Skin lesions		Mucosae	Concurrent neoplastic disease	
Titre	Extensive	Limited	affected	Site	No. of patients
Negative	30	5	6	Chondrosarcoma of scapula	1
				Recurrence of Ca cervix	1
				Ca larynx	1
				Ca thyroid	1
				Ca bronchus	3
				Recurrence of Ca stomach	1
1/10	21	6	5	Ca bladder	1
1/80	14	1	1		
1/160	5	1	1	Ca bladder	1
1/320	14	3	0	Ca bronchus	1
1/640	20	4	2	Ca prostate	1

The youngest case was a female, aged twenty-two, with multiple exostoses (diaphyseal aclasis) since childhood. In February 1978 a right scapuloectomy was carried out for what was reported as a low-grade chondrosarcoma. The possibility of the lesion being a rapidly growing exostosis with a pseudo-malignant appearance could not, however, be discounted. In May 1978 the patient developed a widespread blistering eruption on the face, arms and trunk. A diagnosis of bullous pemphigoid was made by Dr J.J.H.Gilkes, University College Hospital, London, supported by direct immunofluorescence which showed linear BMZ deposits of IgG and IgM. The eruption was controlled by 40 mg prednisolone daily. Unfortunately, the patient now refuses to attend the clinic so further information is unavailable.

Clinicians were also asked about the results of treatment and from their comments it would appear that there were no major differences in the regimes used for the 'seropositive' and 'seronegative' groups, the therapeutic responses of the two groups being similar. Systemic corticosteroids were used alone in forty-nine patients whilst a further forty-nine patients received additional azathioprine. Treatment was considered to have contributed significantly to the death of six patients who died within a few weeks of the diagnosis of pemphigoid being made.

The skin lesions in nine of the twelve patients with concurrent malignant disease cleared promptly with treatment, but in three patients blistering was more difficult to control. In one patient the rash responded to removal of the tumour. A 75-year-old male was found to have a gastric carcinoma which was treated by gastrectomy. Two years five months later he developed a vesiculo-bullous dermatitis herpetiformis-like eruption which on direct IMF showed linear IgG and C_3 deposits in the BMZ of skin; indirect IMF was negative. The rash was partially controlled by topical corticosteroid therapy; systemic treatment was not given. Two months after the diagnosis of bullous pemphigoid was established he developed further gastric symptoms and a recurrence of the tumour was discovered. Successful removal of the neoplasm was followed by clearing of the skin lesions.

COMMENT

The possible association of bullous pemphigoid and malignant disease is still of concern to clinical dermatologists. There are a number of case reports, some dramatic, describing patients with bullous pemphigoid and a malignant neoplasm (Dahl & Ristow, 1978). Chorzelski *et al.* (1978) found malignancy in twelve out of 110 patients with bullous pemphigoid (11%), the expected number of cases in a comparable control group being 0·537 males and 0·293 females. However, other large series of cases have suggested that the relationship is coincidental (Lim, MacDonald & Rook, 1968; Parsons & Savin, 1968; Paslin, 1973; Stone & Schroeter, 1975; Ahmed, Chu & Provost, 1977a; Savin, 1979). Person & Rogers (1977), whilst subscribing to this latter view, found that malignancy was more common in those patients with bullous pemphigoid and negative indirect immunofluorescence findings, 25% of such patients being affected (six out of twenty-six) compared with only 9% of those with positive findings (five out of fifty-five). Our study shows concurrent malignant disease to be present in a similar proportion of 'seronegative' cases (23%) and in even fewer 'seropositive' ones (4%) ($\chi^3 = 7{\cdot}70$ with one degree of freedom, $P > 0{\cdot}01$). It is of interest that five of the eight 'seronegative' cases died from their malignancy within a few months of diagnosis, whereas three of the four 'seropositive' patients are still alive after 16–18 months.

The incidence of mucous membrane involvement in bullous pemphigoid and its possible clinical significance are still uncertain. Rook & Waddington (1953) wrote that it was uncommon and seldom severe, and Ahmed *et al.* (1977b) gave an incidence of 8%. Their findings are contradicted by those of Sneddon & Church (1955) and more recently by Person & Rogers (1977), who found mucosal lesions in more than one-third of cases. Moreover, the frequency of mucosal involvement rose to over 54% in those with negative indirect immunofluorescence findings (Person & Rogers, 1977). Mucosal lesions were present in only fifteen (12%) of our 124 patients, whereas six (17%) of the thirty-five 'seronegative' patients had mucosal involvement. Thus, while the incidence of mucosal involvement in our series is substantially lower than that of Person & Rogers (1977), we found, like them, that mucosal involvement is commoner in the 'seronegative' sub-group, although not significantly so ($\chi^2 = 0{\cdot}60$ with one degree of freedom). Mucosal involvement did not appear to indicate a less favourable therapeutic response or prognosis; only two of the fifteen patients with mucosal lesions had concurrent malignant disease.

The association of bullous pemphigoid and malignant neoplastic disease will undoubtedly continue to be reported and debated. Those who believe that this relationship is coincidental must now explain why malignancy is more common in patients with negative indirect (IgG) immunofluorescence findings. Ideally, dermatologists should continue to search for malignant disease in their pemphigoid patients, and if they find it an attempt should be made to establish a causal mechanism, perhaps using the techniques outlined by Dahl & Ristow (1978).

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Ultraviolet light treatment delays contact sensitization to nitrogen mustard

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SUMMARY

Contact sensitivity to nitrogen mustard was delayed after first treating patients with u.v. light. While the number of patients becoming sensitive to nitrogen mustard after ultraviolet light exposure was not significantly different from the control, the number of patients who became sensitive in the first 30 days was significantly less than the control group. It is postulated that this delay in sensitization is due to an alteration of the patients' epidermal Langerhans cells, produced by the u.v. light exposure.

Nitrogen mustard (mechlorethamine, HN_2) has been shown to be an effective topical treatment for psoriasis (Mandy, Taylor & Halprin, 1971) and mycosis fungoides (Van Scott & Kalmanson, 1973). However, approximately 50% of the patients treated with HN_2 develop delayed contact sensitivity and must either discontinue the therapy or become desensitized to the HN_2 through a lengthy procedure.

Langerhans cells (LCs) have been shown to be instrumental in the development of contact allergies (Stingl *et al.*, 1978; Streilein, Toews & Bergstresser, 1980a) and to be the only epidermal cells that express Ia antigens (Rowden, Lewis & Sullivan, 1977; Klareskog *et al.*, 1977). More recent work has shown that u.v. light exposure decreases the number of LCs demonstrable by ATP-ase staining. This may be the result of either a decreased density of LCs (Streilein *et al.*, 1980a; Streilein *et al.*, 1980b; Bergstresser, Toews & Streilein, 1980) or an alteration of the structure of the LCs (Stingl *et al.*, 1980). In either event, it has been shown that u.v. light exposure inhibits contact sensitivities in mice (Noonan, Pederson & Kripke, 1980), guinea-pigs (Morison & Parrish, 1980) and humans (O'Dell *et al.*, 1980). It seems probable that the effect of the u.v. light on the LCs results in this inhibition of sensitization.

Based on this information, we attempted to prevent sensitization to HN_2 in patients by giving them a course of u.v. light exposure before topical HN_2 therapy. If prevention of sensitization to HN_2 could be induced by this method, it would have obvious clinical implications for the treatment of psoriasis and mycosis fungoides.

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PATIENTS AND METHODS

Fifteen patients were enrolled in the study. Six patients were women and nine were men. Thirteen of the patients had psoriasis, one had mycosis fungoides, and one patient had Kaposi's sarcoma. While the three diseases are different in nature, the rate of contact sensitivity to the HN_2 should be the same. The average age was 56 years with a range of 32–81 years. For a control, a group of twenty-eight male psoriasis patients treated at the Miami Veterans Administration Hospital during 1970–1971 was used. These twenty-eight patients had all been treated with topical HN_2 without u.v. light and followed for one year.

All of the patients in our study received u.v. light exposure. However, the amount, duration and type of radiation varied as shown in Table 1. Each patient received at least one MED before initiation of HN_2 therapy. Three patients were on PUVA therapy and received UV-A while the remaining twelve were exposed to UV-B. A previous study with guinea-pigs showed no difference in the effects of PUVA and UV-B in preventing sensitization to contact allergies (Morison & Parrish, 1980).

Patient 1 started HN_2 therapy immediately following the first UV-B exposure while patients 2 and 3 received one UV-B exposure prior to starting HN_2 therapy the next day; all three continued UV-B treatments for the next 10–20 days. The three patients on PUVA received at least four PUVA treatments (two treatments per week for 2 weeks) before applying the HN_2. Five patients received UV-B treatments twice a day for 5 days and one received exposure twice a day for 3 days. Two patients had been on Goeckerman therapy for the previous 30 days before being switched to HN_2 therapy. After initiating topical HN_2 therapy, the patients applied HN_2 to their lesions (patients with few scattered plaques) or to the whole body twice a day.

RESULTS

All patients were followed for at least two months after initiating topical HN_2 therapy. Only four of the fifteen patients developed a definite sensitivity to HN_2 as proven by patch testing. One of these

TABLE 1. Ultraviolet light teatment with HN_2 therapy

Patient	u.v. light prior to HN_2 (days)	Frequency of u.v. light	Duration of u.v. light+ HN_2 (days)
1	0	b.i.d.	20
2	1	q.d.	14
3	1	q.d.	9
4	7	q.d.	16
5	3	b.i.d.	9
6	PUVA*		Remained on PUVA
7	30	b.i.d.	3
8	30	b.i.d.	0
9	5	b.i.d.	2
10	PUVA*		Stopped PUVA
11	PUVA*		Remained on PUVA
12	5	b.i.d.	15
13	5	b.i.d.	23
14	5	b.i.d.	21
15	7	b.i.d.	21

*At least 2 weeks of twice a week treatments.

four (patient 2) had received only one UV-B exposure prior to initiation of HN_2 therapy. One patient developed a probable sensitivity but this was not confirmed by patch testing. Three patients cleared completely and stopped the use of HN_2 after 5 months, 3 months and 1 month. One patient died from an unrelated cause after having been on the HN_2 for 2 months with no sensitivity. One patient stopped use because it was not effective enough for him and one patient was lost to follow up at 68 days. Four patients are still using HN_2 with good results to control their psoriasis (Table 2).

In our control group of twenty-eight previously treated psoriatic patients, sixteen developed a definite contact sensitivity to HN_2 within 1 year and twelve became sensitive within the first 30 days of treatment. Only five of the u.v. light treated group have become sensitive at this time and only one of the u.v. light treated patients became sensitive within the first 30 days (Table 3). This finding was significant ($P<0{\cdot}01$). The mean time until sensitivity was also increased in the u.v. light treated group. With no u.v. light treatment, the average number of days until sensitivity was 35; in the study group it was 51 (Table 4). If more sensitivities develop within the u.v. light treated group this figure will, of course, increase. At the present time the difference between the mean times is not statistically significant because of the wide range of times and the fact that one group was followed for a year while the study group has not yet been followed for that length of time.

TABLE 2.

Patient	Condition	Sensitive	Duration of HN_2 treatment (days)	Present status
1	Mycosis Fungoides	No	145	Cleared, stopped treatment
2	Psoriasis	No	92	Cleared, stopped treatment
3	Psoriasis	Yes	32	Sensitive, treatment stopped
4	Psoriasis	No	72	Deceased
5	Psoriasis	Probable	70	Stopped treatment, probable allergy
6	Psoriasis	No	130	Still on HN_2 and PUVA
2	Psoriasis	No	68	Lost to follow up
8	Psoriasis	No	31	Stopped treatment, not sensitive
9	Psoriasis	No	115	Still on HN_2
10	Psoriasis	Yes	28	Sensitive, treatment stopped
11	Psoriasis	No	30	Still on HN_2
12	Kaposi's sarcoma	Yes	63	Sensitive, treatment stopped
14	Psoriasis	Yes	63	Sensitive, treatment stopped
15	Psoriasis	No	79	Still on HN_2

TABLE 3. Patients sensitive within first 30 days

	Sensitive	
	Yes	No
Ultraviolet light treated	1	14
Not u.v. light treated	12	16

$\chi^2 = 7{\cdot}6, P<{\cdot}01.$

It is interesting to note that the one patient in the u.v. light treated group who did become sensitive to HN_2 within the first 30 days (patient 10) was one of the three PUVA patients. This patient stopped PUVA therapy upon initiating HN_2 therapy while the other two PUVA patients continued PUVA treatments and both are still on PUVA and topical HN_2 therapy. The additional UV-A received may be a factor in preventing sensitivity in these two patients.

TABLE 4.

	No. of Patients	No Sensitive	% Sensitive	Mean days until sensitive
With u.v. treatments	15	5	33·3	51
No u.v. treatments	28	16	57·1	35

DISCUSSION

While at this time prior u.v.light treatment has not been shown to significantly affect the number of patients developing a contact dermatitis to HN_2, it does significantly delay the time of onset of the sensitivity.

Ultraviolet light has been used to depress systemically the induction of contact dermatitis in mice to trinitrochlorobenzene (TNCB) and dinitrofluorobenzene (DNFB) (Noonan *et al.*, 1980). The suppression was directly related to the log of the u.v. dose. Morison and Parrish (1980) using a guinea-pig model exposed a 6 × 7 cm. region of the animal to UV-B for 7 days before immunization and 7 days between immunizations with dinitrophenol-bovine γ globulin (DNP-BγG) and induced local and systemic suppression of delayed hypersensitivity to DNP-BγG.

In a human study, O'Dell *et al.* (1980) sensitized volunteers to 2,4 dinitrochlorobenzene (DNCB). Higher concentrations of DNCB were needed to elicit positive patch test reactions on the sun-damaged skin of the neck than on the protected skin of the upper back. There was no difference between sun-damaged and non-damaged skin in response to a primary irritant. Response to intradermally injected *Candida*, mumps and tuberculin antigens were also less intense in sun-damaged skin than in sun-protected skin.

Ultraviolet radiation suppressed cell mediated immunity. In order to determine if this was an epidermal or dermal effect, a group at the National Cancer Institute (Noonan *et al.*, 1980) removed the epidermis of mouse skin pretreated with an FS40 sun lamp and exposed it to TNCB and DNFB. Removal of the epidermis after irradiation prevented the previously observed tolerance. Lymphoid cell transfer studies showed that the suppression was associated with antigen specific suppressor lymphocyte development. They concluded that u.v. exposure of the epidermis of mice suppresses the immune response to contact allergens.

In the epidermis an immunocompetent cell that plays a role in delayed hypersensitivity is the LC. Bergstresser, Fletcher & Streilein (1980) found a relationship between decreased LC surface density and immunological tolerance. LCs were absent from the corneas of the guinea-pig, hamster and mouse. Fewer LCs were found in the hamster cheek pouch than other areas of the hamster. The cornea and the hamster cheek pouch are sites where grafts or tumours often survive and thus are 'immunologically privileged.' Bergstresser's group hypothesized that LC deficiency permits antigen to enter the epithelium without recognition.

Streilein *et al.* (1980b) showed that UV-B irradiation reduced the number of LC cells (ATP-ase positive cells) transiently in mice. They demonstrated a direct correlation between the return of LC density toward normal and the increased ability of the skin to allow DNFB sensitization. Fifteen days after cessation of UV-B exposure LC density returned to normal and the development of DNFB sensitization was permitted.

Noonan's group (1980) found that in DNFB or TNCB sensitization, anergy did not develop when mice were sensitized one day after UV-B, but appeared by the third day and persisted for a minimum of 2 weeks. It may take a minimum of three days for UV-B to affect the LCs ability to recognise antigens.

The delay of onset of contact allergy to HN_2 with u.v. light pretreatment found in our study may be due to interference with normal LC function. However, this u.v. light-induced interference is transient (i.e. 15 days in the mouse). It appears that LCs are continuously being replenished from a pool of precursor cells which originate in the bone marrow (Tamaki, Stingl & Katz, 1980). 'Booster' u.v.light therapy may be necessary to ensure continued prevention of sensitization. The delayed but ultimate sensitization of our patients may be the result of the absence of such a 'booster' and the recurrence of functional LCs.

DuVivier & Vollum (1980) did not find that continued PUVA treatments prevented sensitivity in a study they did with mycosis fungoides patients. In their study three out of five patients being treated with PUVA and HN_2 did develop a contact allergy to HN_2. However, four of these five patients started PUVA therapy and HN_2 therapy simultaneously; they had received no pretreatment with u.v. light. The one patient who had been on previous PUVA therapy did become sensitive but no information was given as to when the last PUVA treatment before starting $PUVA+HN_2$ therapy was given. This pretreatment period with u.v. light may be necessary to prevent sensitization. In our study the two psoriasis patients who remained on PUVA have not become sensitive. They had received pretreatment with u.v. light. We believe that more work with a larger number of patients needs to be done to resolve this.

We consider this to be a preliminary study and plan to change our protocol to include 'booster' u.v. light at intervals to our patients. The time between these 'boosters' has yet to be determined but a 2–4 week interval seems likely. Likewise, the number of u.v. light treatments necessary in this booster has yet to be determined. However, it does appear that for the initial u.v. light treatment, 5 days is adequate. We hope that this preliminary study will help bring a theoretical concept to a practical application.

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Pharmacology and Treatment

The atrophogenic effect of crude coal tar on human epidermis

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SUMMARY

The effects of crude coal tar on human epidermis were studied in fourteen healthy young adult males. A 20% reduction in viable epidermal thickness, as determined by computer assisted analytical microscopy, was observed after 40 days of topical treatment. Prior to this long term effect of crude coal tar, an initial transient hyperplasia was observed during the first 2 weeks of treatment. These findings indicate that crude coal tar by itself can act as a cytostatic agent on normal human skin, when applied intensively.

In a recent study in which the effects of coal tar and vitamin A acid on membrane coating granules were analyzed (Lavker, Grove & Leyden, 1979), we gained the impression that the epidermis was thinner after the tar treatment. This was a paradoxical result for coal tar is known to produce hyperplasia and hyperkeratosis in man (Jambon, 1909; Combes, 1943, 1944; Kaidbey & Kligman, 1974) and animals (Sarkany & Gaylarde, 1976; Elgjo & Larsen, 1973).

We therefore decided to re-examine the effects of crude coal tar (CCT) on human epidermis using computer assisted analytical microscopy (Grove, Lavker & Kligman, 1978; Grove, 1980), and found that tar could indeed lead to epidermal atrophy after long term use.

MATERIALS AND METHODS

Fourteen healthy young adult males served as volunteers. 5% crude coal tar (CCT) in Hydrophilic ointment, U.S.P. was applied once daily for 40 days to the suprascapular area. To prevent soiling of the clothes, the site was covered with a square of gauze, taped around the edges. A control site was similarly non-occlusively covered. At the end, thin slice biopsies were taken under local anaesthesia and processed for light microscopy. Great care was taken that the 7 μm sections were cut perpendicular to the surface.

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TABLE 1. Effects of Hydrophilic ointment U.S.P. on the viable epidermal thickness (VET)*

			Control	Hydrophilic ointment
Patient	Age	Race	VET	VET
1	19	W	48·1±6·5	44·7±6.6
2	30	B	64·3±13·3	62·8±14·3
3	32	W	42·3±4·0	45·2±6·2
4	24	W	57·6±7·6	58·9±7·1
5	22	W	46·7±7·2	45·5±3·8

* VET expressed as arbitrary units.

Our prior experience with Hydrophilic ointment U.S.P. indicated that this vehicle was inert with regard to changing skin thickness. To confirm whether or not this was true over a 40 day period, five individuals were treated with Hydrophilic ointment. As can be seen in Table 1, this vehicle had no effect on viable epidermal thickness.

Histometric measurements were made with a Magiscan Image Analyzing Computer as previously described (Grove, 1980). Briefly, a high performance television camera is used to obtain an electronic image through a microscope. These signals are then digitized and processed in an image analyzer interfaced to a mini-computer. The operator can extract from the array of picture points the features of special interest and reject everything else by standard software programs. The processed image is reconverted and displayed on a video monitor so that the operator can compare it with the original, assuring that the measurements are relevant. By using an Editor light pen, the operator can interface himself into the system to further select designated regions.

In this manner, we were able to determine the area occupied by the viable epidermis per standard low power frame. Since this is essentially an integrated value of the area over the undulating dermal–epidermal junction, we have a much better estimate of viable epidermal thickness (VET) than the conventional method of linear point measurements can provide (Grove *et al.*, 1978; Grove 1980).

To obtain more detailed information on the kinetics of the response to CCT, biopsies of treated and control sites were obtained every 2 weeks over an 8 week period.

The paired *t*-test was used to analyze these data.

RESULTS

Typical histological profiles of CCT and control specimens after 40 days of treatment are shown in Fig. 1. The CCT treated epidermis was thinner than the control. However, the stratum corneum was obviously thicker and had more cell layers. The epidermal cells in the CCT specimens seemed morphologically normal without any obvious change in cell size. An increase in the amount of keratohyalin granules was observed after treatment with CCT.

By histiometric analysis (Table 2) CCT caused a decrease in the viable epidermis in every case, averaging 19·2%. A paired *t*-comparison showed this to be a highly significant difference ($P<{\cdot}001$).

By histiometric study of specimens taken every 2 weeks, the epidermis was thicker by 13·3% by 2 weeks, returned to the baseline by 4 weeks, was decreased by 23% after 6 weeks and still further reduced by 8 weeks (34%) (Fig. 2).

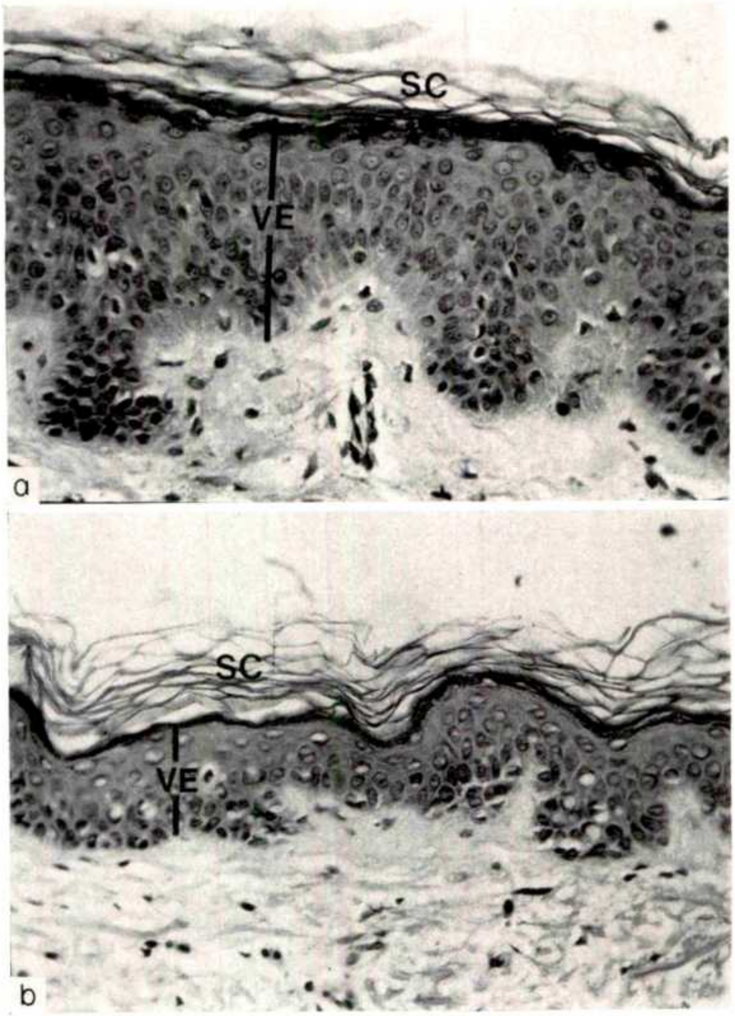

FIGURE 1. Light micrograph of control epidermis (A) and epidermis 40 days after treatment with crude coal tar (B). Crude coal tar treatment results in a thinner viable epidermis (VE) and a thicker stratum corneum (SC) than the control (× 200).

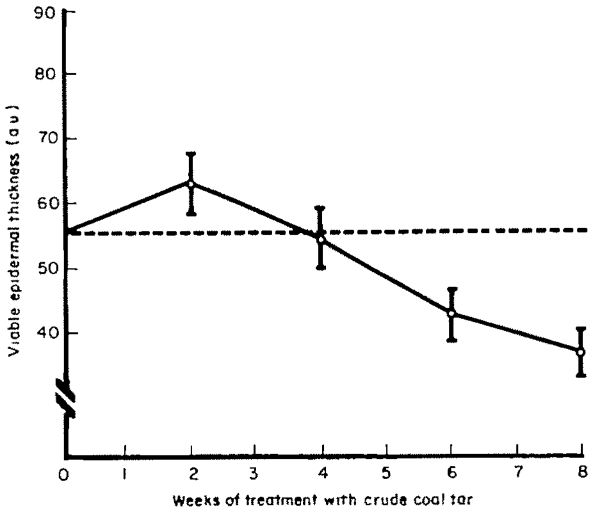

FIGURE 2. Kinetics of the response of the epidermis to crude coal tar. Viable epidermal thickness measurements were made on crude coal tar treated specimens taken every 2 weeks for 8 weeks. – – – viable epidermal thickness of control specimens.

TABLE 2. Effects of crude coal tar on human epidermis

Subject	Age	Race	Viable epidermal thickness*		
			Control	Crude coal tar	Δ%
1	19	W	48·1±6·5	39·4±6·8	−18·1
2	30	B	64·3±13·3	50·0±8·1	−22·1
3	32	W	42·3±4·0	37·5±5·4	−11·4
4	24	W	57·6±7·6	45·1±7·2	−21·9
5	22	W	46·7±7·2	36·2±3·6	−22·5
6	22	W	42·5±7.9	41·4±8·4	−2·6
7	19	B	53·5±12·0	42·6±9·6	−20·3
8	28	W	58·9±11.6	51·1±9·2	−13·3
9	19	W	60·4±11·6	46·2±10·4	−23·5
10	22	B	78·7±13·8	60·9±17·3	−22·6
11	19	B	64·7±11·6	45·7±4·4	−29·3
12	20	W	61·3±5·8	40·3±7·0	−34·3
13	20	W	48·8±8·9	45·5±8·4	−8·8
14	22	W	42·3±10·1	34·8±6·5	−18·9
	Average		55·0±10·6	44·1±6·9	−19·2

* Viable epidermal thickness expressed as arbitrary units.

DISCUSSION

The long-term topical application of CCT to normal human skin clearly decreases the thickness of the viable epidermis, unaccompanied by signs of cell injury or shrinkage. This finding is at variance with the widespread belief that CCT induces epidermal hyperplasia. For example, Combes (1943, 1944) stated that the characteristic effect of CCT was acanthosis. Likewise, Kaidbey & Kligman (1974) in their study of coal tar acne found that the epidermis was acanthotic a few weeks after its application to the backs of adult males. Furthermore, Jambon (1909) observed an increased mitotic rate when tar was applied to normal human skin. Epidermal hyperplasia has also been found in hairless mice (Elgjo & Larsen, 1973) and guinea-pigs (Sarkany & Gaylarde, 1976). However, in all of these studies the duration was short (4 weeks or less). We too found acanthosis but only in the first couple of weeks. This is probably due to irritation and is the non-specific response produced by any primary irritant. By 6 weeks, however, the epidermis begins to become atrophic with an even greater thinning by 8 weeks. Irritants do not behave in this paradoxical way. We consider that the initial hyperplasia obscures the true pharmacological activity of tar, which is in fact an atrophogenic effect.

Coal tar has for centuries been a major therapeutic agent in the treatment of psoriasis, especially when combined with ultra-violet light (Goeckerman, 1925). This has always seemed paradoxical because psoriasis is a hyperproliferative disease with a markedly thickened epidermis. Up till now, coal tar was thought to induce a histological picture rather similar to psoriasis. A physician from another world would never choose tar to treat psoriasis. The most effective modern therapeutic agents, exemplified by corticosteroids, are cytostatic when used intensively. Of course tar differs from steroids in a number of particulars but its beneficial effect in psoriasis is no longer surprising. Indeed, in hairless mice, coal tar has been shown to inhibit DNA synthesis by direct measurement (Stoughton, DeQuoy & Walter, 1978). This is concordant with our finding and places tar among a number of disparate agents which have cytostatic effects.

Another interesting feature of CCT is the presence of a hyperkeratotic horny layer despite a thinned epidermis. Evidently this represents retention hyperkeratosis in which the horny cells fail to detach from each other. This type of pattern is quite often seen in the epidermis from old skin (Montagna, 1965; Lavker, 1979) and in the follicular epithelium of comedones (Kligman, 1974). The production of this type of epidermis is further evidence of the uniqueness of tar, for the majority of irritants increase the thickness of the horny layer by stimulating mitosis, inevitably ending in hyperplasia not hypoplasia.

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Disodium cromoglycate in dermatitis herpetiformis

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SUMMARY

Eleven patients with dermatitis herpetiformis, all requiring dapsone to control their rash and taking a normal diet, were given disodium cromoglycate (DSCG) 1·5–1·6 g daily. Eight out of the eleven patients continued to take DSCG for periods varying from 6–11 months, the other three patients chose to discontinue the DSCG before 6 months. Of the eight patients taking DSCG for at least 6 months, none was able to stop taking dapsone. In three of the eight, the dapsone requirements were unaltered, whilst in two it decreased and in three it increased. The mean daily dose of dapsone was 105 mg/day before DSCG and 141 mg/day after DSCG.

In the eight patients who took DSCG for at least 6 months, intestinal biopsies were performed before and after this drug. The macroscopic appearance was unchanged in four, improved in two and worse in two. The mean interepithelial lymphocyte count was 346 before and 342 after DSG.

Disodium cromoglycate (DSCG) has been claimed to be helpful in ulcerative colitis (Heatley *et al.*, 1975), Crohn's disease (Henderson & Hishon, 1978) and food allergies (Vaz, Tan & Gerrard, 1978), although the first two claims have been disputed (Bucknell *et al.*, 1978; Grundman, Williams & Turnberg, 1978). The rash of dermatitis herpetiformis (DH) is gluten dependent and is associated with a gluten sensitive enteropathy. A recent report by Rhodes (1978) claimed that oral DSCG was helpful in controlling the rash of DH, but no attempt was made to evaluate the effect of DSCG on the intestinal lesion associated with DH. In this study the effect of DSCG on both the intestinal and skin lesions is reported.

PATIENTS AND METHODS

Eleven patients were initially selected but only eight completed the study. There were five males and three females and their ages ranged from 38 to 63 years. The diagnosis of DH was made by the response of the rash to dapsone and the presence of IgA in the dermal papillae of the clinically uninvolved skin. At the time of study, all patients were taking a normal diet and all required dapsone to control their rash.

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Patients were instructed to take the lowest possible dose of dapsone to control their rash prior to commencing DSCG. DSCG was initially given at a dose of 1·5 g daily, consisting of fifteen 100 mg capsules. Patients were told to divide the dose and take the drug 15–30 min prior to food. Towards the end of the study, the dose of DSCG was 1·6 g daily, consisting of eight 200 mg tablets. These were also taken in divided doses at the same time, prior to food.

Patients were seen approximately every 2 months during the study and asked to reduce their dose of dapsone prior to each visit, to see if their dapsone requirements were decreasing to control their rash. Initially it was decided to study the patients for a 6-month period, and this was the minimum period, but a number were followed for longer periods, up to 11 months.

All patients had a small intestinal biopsy prior to starting DSCG and another biopsy at the end of the study period. The biopsies were assessed by their macroscopic appearance and the inter-epithelial lymphocyte count (IELC) (Fry *et al.*, 1972).

RESULTS

Three out of the eleven patients failed to take the DSCG for up to 6 months. There were no specific side-effects but the patients stated a dislike of having to take such a large number of capsules per day. The results of the remaining eight patients were analysed.

Dapsone requirements (Table 1)

In three of the eight patients the dapsone requirements were unaltered, three patients required a higher dose, and two a lower dose. None of the patients was able to stop dapsone. In patient no. 8 the dapsone requirements had fallen gradually in the previous 2 years and in the period under study there was a further fall. As a group, the mean dose of dapsone was higher (141 mg) after DSCG than before taking this drug (105 mg).

Intestinal biopsies (Table 1)

After DSCG the macroscopic appearance of the intestinal mucosa was the same in four patients, showed improvement in two and was worse in two.

TABLE 1. Dapsone requirements and intestinal biopsies before and after DSCG

Patient no.	Age (years)	Sex	Dapsone requirements (mg/day)		Intestinal biopsy			
					Macroscopic appearance		IELC	
			Before	After	Before	After	Before	After
1	43	M	300	600	Convoluted	Convoluted	374	521
2	63	M	100	100	Leaves	Flat	278	406
3	45	M	100	150	Fingers and leaves	Leaves	332	459
4	61	M	100	100	Convoluted	Leaves	362	220
5	38	M	100	50	Flat	Flat	384	184
6	41	F	50	50	Leaves	Fingers and leaves	271	183
7	58	F	43	71	Convoluted	Convoluted	434	392
8	43	F	43	14	Flat	Flat	329	368

The IELC was increased in four patients and decreased in four patients. The mean count was virtually the same before (346) and after DSCG (342). In the two patients who showed an improvement in the macroscopic appearance of the mucosa (Nos. 4 and 6), the IELC also improved, whilst in the two patients (Nos. 2 and 3) in whom the macroscopic appearance showed more abnormality after DSCG, the IELC showed an increase.

DISCUSSION

These results show that, after periods varying from 6–11 months, DSCG does not enable patients with DH to reduce their dapsone requirements for control of the rash. These results are at variance with those of Rhodes (1978) in which she reported that four out of fifteen patients were able to discontinue dapsone and another nine of these fifteen patients were able to reduce their dose of dapsone. The time taken to obtain reduction of dapsone requirements with a gluten-free diet is usually 6 months, but may on occasions be longer (Fry *et al.*, 1968, 1973). Assuming that DSCG may have a beneficial action in DH then it may be reasonable to expect the dapsone requirements to fall within 6 months, and in fact Rhodes claimed a reduction of dapsone requirements within one month. If DSCG had an effect on dapsone requirements, then stopping DSCG should result in a recurrence of the rash; this obvious and crucial test was not carried out by Rhodes in her study. It is possible that not all the patients studied by Rhodes had DH, as the presence of IgA in the uninvolved skin was not used as a criterion for diagnosis. In addition, it is not stated that patients were instructed to reduce their dapsone requirements prior to DSCG to be absolutely certain of the minimum requirements. Spontaneous remissions, although rare, may occur in patients with DH (Alexander, 1975; Fry *et al.*, 1980) and these will be missed if patients are not told to reduce their dapsone requirements at regular intervals if their skin rash is well controlled. Therefore it is possible that Rhodes' patients were taking dapsone unnecessarily prior to being given DSCG.

In two of the eight patients after DSCG, there was improvement in the macroscopic appearance of the intestinal biopsy, and this was accompanied by a fall in the IELC in both patients. However, in another two patients after DSCG the macroscopic appearance showed deterioration and this was accompanied by a higher IELC.

It would seem most likely that these changes in the intestinal biopsies after DSCG are best explained by the patchy nature of the enteropathy which is known to occur in DH (Brow *et al.*, 1971), and therefore it is likely that a proportion of patients will show some change, either improvement or deterioration, when biopsied on different occasions.

In the two patients whose intestinal biopsies improved, their dapsone requirements were the same after DSCG, whilst in the two whose biopsies deteriorated, the requirements were the same in one and increased in one.

It is generally accepted that DSCG is poorly absorbed (Walker *et al.*, 1972) and therefore any action that DSCG may have in DH would probably be on the intestinal mucosa. It has been postulated that DSCG may exert beneficial action in 'allergic' states by its action on mast cells. These cells are not a feature of gluten sensitive enteropathy, the cellular infiltrate being mainly lymphocytes and plasma cells.

Although many patients find a gluten-free diet difficult to adhere to, it is arguable whether taking tablets before each meal is any better. The reason for our three patients defaulting from the study was the dislike of taking fifteen capsules a day, and in our hands with no obvious benefit to their problem.

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D-Penicillamine in the treatment of keloids

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SUMMARY

Long term low-dosage penicillamine has been shown to be ineffective in two prospective trials of its efficacy in the treatment of keloids.

Penicillamine is an amine with diverse metabolic effects. It has been used as a chelating agent to rid the liver and brain of copper, as a sulphydryl donor and as a possible immunosuppressive. Pencillamine also inhibits collagen maturation (Herbert *et al.*, 1974).

Collagen is secreted from fibroblasts as procollagen, which rapidly polymerises to form a collagen fibre. The fibre is then stabilised by the formation of intermolecular and intramolecular crosslinks which confer on collagen its characteristic stability and inelasticity. D-Penicillamine acts by preventing the formation of aldehyde crosslinks.

The quantity of reducible crosslinks relative to total collagen is highest in adolescence and childhood and declines rapidly thereafter. These crosslinks are presumed, but not proven, to change with increasing age to some currently unidentified mature crosslinks. There are certain tissue differences in the pattern of reducible crosslinks which depend upon the degree of lysine hydroxylation. Collagen solubility refers to the extractability of tissue collagens with various salt and acidic solutions, and extractability varies according to the crosslink pattern of the particular tissue. Generally salt extractable collagens are uncrosslinked and acid extractable collagens have a monohydroxy crosslink.

Keloids have a high proportion of insoluble collagen which implies only that the crosslinks are different from those of mature skin. They contain a high proportion of the reducible keto-crosslink which in contrast to the crosslink in normal newly synthesized skin is resistant to D-penicillamine (Bailey *et al.*, 1975). However, Moynahan (1973) claimed that penicillamine was valuable in the treatment of morphoea when used in low dosage over a long time. He claimed similar effectiveness in the treatment of hypertrophic burns and keloids (Moynahan 1974, 1977) and this supports the work of Copenhagen (1968).

PATIENTS AND METHODS

In the first study, which was controlled, we compared the use of systemic penicillamine with local steroid injection in the treatment of earlobe keloids. There were three groups of fifteen patients, each

The work was carried out at the Regional Plastic and Jaw Surgery Centre, Mount Vernon Hospital, Middlesex.

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with bilateral keloids. The groups were matched for age (range 2–42 years) and race. In all groups the keloids were completely excised including the epithelialised track. The defects were repaired with 6/0 nylon sutures which were removed after 4 days. In the first group, the patients also received oral penicillamine in the form of one 250 mg capsule daily for 3 months. If there was a recurrence, the treatment was increased to 500 mg daily and continued for 6 months. In the second group, triamcinolone (5 mg) was injected subcutaneously into the wound edges at the time of excision, and again on three further occasions at monthly intervals. In the third group, the control group, excision was the only form of treatment. The patients were followed up for 2 years.

In a second study, which was uncontrolled, we treated twelve patients, with a variety of keloids, hypertrophic scars and contractures. They received a similar low dosage, long-term regime of penicillamine orally. Ten patients had keloids of the shoulder, pre-sternal area, back, perineum or face, and in seven of these patients the keloids were multiple. Two patients were treated by excision and suture and eight by excision and skin grafting. All ten received penicillamine for 4–12 months, the treatment continuing at a higher dosage, 500 mg daily, until a definite recurrence had been observed for at least 3 months. The remaining two patients had burns. One suffered full thickness acid burns of the face and neck and was treated prophylactically beginning 2 weeks after the injury. A second patient also had acid burns of the face and was treated 2 years after the injury for severe itching of hypertrophic scars in the beard area.

RESULTS

In the first study, four patients who had earlobe keloids excised had a unilateral recurrence. Three of these had received penicillamine and one had received triamcinolone. There were no recurrences in the control group. There was no significant difference in recurrence rate between the three treatment groups ($\chi^2 = 3{\cdot}8$, $P>0{\cdot}1$).

In the second study of ten patients with keloids at sites other than the earlobe, nine developed a recurrence and the tenth had a poor scar. Six of these patients required further treatment of their keloids. Seven patients in this group with keloids elsewhere, which were not surgically treated, were also unchanged during the course of oral penicillamine. Penicillamine was also ineffective in the two patients with burns, and both patients required further surgery.

DISCUSSION

One can conclude from this study that low dosage, long term penicillamine treatment of keloids is ineffective. Penicillamine is relatively safe in this dosage, but two of our patients developed loss of taste and one developed a rash. However, high dosage is accompanied by more serious side effects and these preclude its recommendation for the treatment of keloids.

The results of these studies are at variance with the work of Copenhagen (1968) and Moynahan (1974, 1977). Copenhagen was forced to discontinue the use of penicillamine in the treatment of keloids due to the high incidence of serious side effects from the drug which was presumably given at a higher dosage. However, Moynahan using the same dosage as in this study claimed even better results in the penicillamine treatment of keloids than in the treatment of morphoea. He did not give clinical details of his patients but it would seem that many of them suffered from the hypertrophic scars caused by burns. This type of scar hypertrophy, particularly the dramatic hypertrophy resulting from a deep dermal burn in a child, tends to regress spontaneously over a period of two or three years. Maybe the penicillamine treatment of some of his patients coincided with this period of natural regression. Moynahan did not perform controlled trials in the treatment of either keloids or morphoea on the grounds that the treatment was so effective that the drug could not be reasonably withheld.

A most interesting finding in these studies was that keloids of the earlobe rarely recurred after excision, while those elsewhere almost invariably recurred. Further work is being carried out, to try to elucidate the factors involved.

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Six-month controlled study of effect of desoximetasone and betamethasone 17-valerate on the pituitary-adrenal axis

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SUMMARY

Twenty-two patients were treated with desoximetasone emollient cream 0·25% twice daily without occlusion for 6 months. Patients applied the medication to approximately one-third of their body over psoriatic lesions. Corticosteroid plasma cortisol values decreased to below normal limits in nine patients before the 6-month study was terminated. In four of these the plasma cortisol spontaneously returned to normal despite therapy; in four other patients, however, the plasma cortisol was still suppressed at the end of 5 months of continual therapy but returned to normal within 7 days of discontinuation of the medication. In one patient, lost to further follow-up at 5½ months of therapy, the trend at the fourth month was an increase in plasma cortisol to within one unit of normal range. Betamethasone 17-valerate 0·1% cream applied twice daily did not suppress plasma cortisol in twenty-three patients similarly tested. The clinical response to desoximetasone emollient cream was significantly better than to betamethasone valerate cream. This study closely approximates the way in which many patients with steroid-responsive dermatoses use potent topical steroids, namely over a long time period and without occlusion.

Many new and potent topical steroids have become available to the practising dermatologist. The potency of a steroid seems to parallel incidence and severity of its side effects such as striae, atrophy, perioral dermatitis, acne and exacerbation of rosacea. Additionally, topical steroids used around the eyelids have been implicated as a contributory factor of glaucoma (Cubey, 1976). Side effects of the new potent topical steroids may not be confined to the skin. Such steroids may affect the pituitary-adrenal axis with subsequent suppression of adrenal function (Carruthers, August & Staughton, 1975; Himathongkam *et al.*, 1978). To assess the efficacy of a new glucocorticosteroid, desoximetasone

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emollient cream 0·25% as compared to betamethasone 17-valerate cream 0·1%, and to determine whether these steroids suppress the pituitary-adrenal axis, we administered these drugs to forty-six psoriatic patients over a 6-month time course.

PATIENTS AND METHODS

Forty-six adult patients, under treatment for psoriasis at Scripps Clinic and Research Foundation, La Jolla, California, volunteered for our study. These patients had lesions covering 20–60% of the body area. This study excluded pregnant patients. Patients with pustular psoriasis or psoriasis of the palms or soles were excluded.

Patients were kept off topical steroids for at least 14 days and systemic steroids or antimetabolites for at least 1 month before entering the study. Medications other than those prescribed for psoriasis were continued.

Prior to treatment, each patient was subjected to a complete physical examination at which time pruritus, erythema, scaling and induration were graded on a 1 (no involvement) to 5 (very severe involvement) scale. Complete blood count, SMA-12, urinalyses, plasma cortisol levels, and urinary 17-hydroxycorticosteroids determinations (Van der Vies, 1961) were also made. Patients (non-fasting) were seen between 7:00 a.m. and 9:00 a.m.; each patient came in at the same time each visit so that conditions for comparison between baseline and succeeding plasma cortisol and urinary 17-hydroxycorticosteroid levels would be as constant as possible. Of the forty-six patients, twenty-three were randomly selected to receive desoximetasone and the other twenty-three received betamethasone.

At the first examination, each patient was assigned a patient number and given eight 45 g tubes of betamethasone 17-valerate cream 0·1% or twenty 20 g tubes of desoximetasone emollient cream 0·25% by an investigator; all tubes contained identical labels indicating patient number. The patients were instructed to apply the test material lightly twice daily as a thin film to the psoriatic lesions avoiding intertriginous areas and the scalp. No occlusive dressings were used. At the time of each visit (at the end of months 1–6), the patient brought in any unused tubes of medication to the first investigator who tabulated the number of tubes used each month by the patient and issued additional medication for the next 4 weeks. A second investigator monitored each patient for overall changes from baseline evaluations and side effects, evaluated response to therapy and supervised blood and urinalyses at months 1, 2, 4 and 6. Thus, neither the patient nor the second investigator knew which of the two products the patient received. Each patient was given a complete physical examination at the end of the sixth month. For those patients who had abnormal (normal = 5·0–20·2 μg/100 ml plasma) plasma cortisol levels at the sixth month evaluation plasma cortisol levels were redetermined 1 week after cessation of treatment.

RESULTS

Of the forty-six patients entered into our study, forty-three completed the treatment protocol. One patient from the desoximetasone group discontinued the protocol at the end of 21 days because the substantial exacerbation of his psoriasis necessitated oral medication; his plasma cortisol levels were abnormal (2 μg/100 ml plasma) at this time; he was not included in the statistical analysis. Due to personal reasons, patient 9 in the desoximetasone group discontinued the protocol after 5¼ months; however, since his plasma cortisol level was determined 4 days after treatment ended, his data are included in the analysis. One patient in the betamethasone valerate group did not return for her 6-month evaluation; nevertheless, she was included in the analysis based on observations estimated from the previous months, values and clinical evaluations. Thus, there were twenty-two patients (twelve males and ten females) in the desoximetasone group and twenty-three (twelve males and eleven females) in the betamethasone 17-valerate group whose data were compared. They were all Caucasian. The average ages were 51 years (range 17—74 years) for the desoximetasone group and 45 years (range 17–70 years) for the betamethasone 17-valerate group. There were no significant differences between the two treatment groups with respect to sex, age, disease status and duration of the dermatosis.

No significant differences between the two drugs were detected, using the contingency table analysis for any haematological, SMA–12 (including glucose) or urinalysis variable for any time period for any individual patient or between the treatment groups.

The twenty-three patients on the betamethasone 17-valerate 0·1% cream protocol averaged 11·0 g/day of betamethasone 17-valerate cream (range 4·9–12·8 g). Clinically 34% of patients improved over 50%, and 66% less than 50% after 6 months of therapy. There was no evidence of suppression of the pituitary-adrenal axis in any of the twenty-three patients using betamethasone valerate over the 6-month period (Table 1).

TABLE 1. Summary of patients showing adrenal suppression and clinical response

	Betamethasone valerate 0·1%		Desoximetasone 0·25%	
	After 1 month	After 6 months (end of study)	After 1 month*	After 6 months† (end of study)
No. of patients	23		22	
Adrenal suppression (no. of patients showing suppression any time during 6-month study)	0		9‡	
Clinical response				
Excellent (> 75% improvement)	1 (4%)	1 (4%)	8 (36%)	8 (36%)
Good (50–75% improvement)	7 (30%)	7 (30%)	4 (18%)	8 (36%)
Other (less than 50% improvement)	15 (66%)	15 (66%)	10 (46%)	6 (28%)

*P = 0·03 in favour of desoximetasone.

†P = 0·009 in favour of desoximetasone.

‡In four of the nine, plasma cortisol returned to normal levels during treatment and in an additional four patients, plasma cortisol returned to normal levels within 1 week following treatment. (In one patient who was lost to further follow-up, the trend at the fourth month of evaluation was a gradual increase in plasma cortisol to within one unit of normal range.)

The twenty-two patients begun on desoximetasone 0·25% emollient cream averaged 9·8 g daily of desoximetasone emollient cream (range 2·3–14·3 g). Clinically 72% of patients improved over 50%, and 28% less than 50% after 6 months of therapy. Nine of the twenty-two patients who received desoximetasone had decreased plasma cortisol levels (Table 2). Four of these patients had levels that returned to normal during the study despite continuation of the drug at the extreme of the dosage range. For four other patients, the plasma cortisol levels were below normal limits at the end of 6 months of continuous therapy; 1 week later plasma cortisol tests were repeated for these patients, and the values had returned to normal. Patient 9 who was last examined at 5½ months of treatment had cortisol levels below normal limits; but the trend on the fourth day after discontinuation of desoximetasone was a gradual increase in plasma cortisol to within one unit of normal range so it has been assumed that his levels have normalized. As a group, these nine patients applied more medication

TABLE 2. Patients receiving desoximetasone cream 0·25% with low plasma cortisol during the study

Patient	Pre-treatment	Month 1	Month 2	Month 4	Month 6		g/day used topically month prior to low value	Clinical response at* months of lowest value
1	10·0	7·0	4·0†‡	9·0	7·0		14·3	Good
2	13·0	2·0‡	8·0	7·0	9·0		14·3	Excellent
3	9·0	6·0	3·0‡	11·0	10·0		10·7	Other
4	9·0	6·0	3·0‡	5·0	5·0		14·3	Excellent
						Follow-up*	Average g/day used over 6 months	Clinical response 6 months
5	6·0	2·0‡	3·0‡	4·0	4·0‡	7·0	14·3	Excellent
6	8·0	6·0	4·0‡	5·0	3·0‡	11·0	10·0	Good
7	8·0	10·0	6·0	8·0	2·0‡	6·0	11·4	Good
8	10·0	4·0‡	5·0	4·0	3·0‡	13·0	8·6	Good
9	7·0	2·0‡	2·0‡	2·0	3·0‡	4·0‡	14·3	Excellent

*Excellent (<75% improvement); good (50–75% improvement); other (less than 50% improvement).

†Follow-up plasma cortisol 7 days after study terminated in all subjects except subject 9 who moved out of area due to emergency job transfer—his end of study plasma cortisol was at 5¼ months; repeat plasma cortisol 4 days later.

‡Below normal range.

daily than the other thirteen patients treated with desoximetasone; however, of those thirteen patients, seven applied the same amount of desoximetasone emollient cream daily as those nine patients but did not show plasma cortisol suppression. All but one, patient 3, of the nine patients who had suppressed cortisol levels during or at the end of the study had over 50% improvement of their skin; this patient had suppressed plasma cortisol levels during the second month which normalized during the fourth month.

DISCUSSION

Our efforts to help to resolve the controversy as to whether topical glucocorticosteroids cause adrenal insufficiency when used without occlusion over a long time period have clearly demonstrated that these drugs may in fact precipitate decreased plasma cortisol levels in some individuals, and at times decrease levels below normal range. However, these decreases are likely to be reversed while the patient continues medication, and almost certainly reversed by discontinuation of medication. The predilection for decreases in plasma cortisol levels seems to be dependent upon the type of medication used and to be dose-dependent. This is demonstrated by the fact that our patients who received betamethasone did not manifest decreased plasma cortisol levels, although more than one-third of the patients on desoximetasone did have such decreases. Furthermore, the patients on desoximetasone who had these decreases were using relatively more medication than the other patients in this group.

It thus seems probable that the diverse results of investigators (Carr & Tarnowski, 1968; Wilson, Williams & Marsh, 1973; Goldman & Cohen, 1962; Rostenberg, 1961; March & Kerbel, 1964; Munro & Clift, 1973) who were unable to demonstrate plasma cortisol decreases related to topical corticoids as compared to Scoggins (1962), Gill & Baxter (1964), Scoggins & Kliman (1965) and Gomez, Kaminester & Frost (1977), whose findings were similar to ours, can be accounted for by inconsistent variables in the different studies. These variables are the specific medications used, the dosage, how liberally the medication is applied, what areas are exposed to medication, whether or not occlusion is used in conjunction with topical corticosteroids, and the amount of time the patient is on treatment.

The fact that we and others have found decreased plasma cortisol levels in correlation with topical steroid therapy should serve as a warning that at the very least some of these drugs do influence the pituitary-adrenal axis. The extent of the influence and the implications can only be speculated upon at this time, since plasma cortisol levels only indicate basal homeostasis and are not indicators of the functional integrity of the adrenal gland or its reserves. Until these determinations can be made, the less potent corticosteroids should be the mainstay of treatment for dermatoses. The more potent medications should only be used when other measures have failed.

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Case Reports

Juvenile xanthogranuloma with recurrent subdural effusions

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SUMMARY

A 2-year-old girl with extensive cutaneous xanthogranuloma and recurrent subdural effusions has been investigated. Differentiation from histiocytosis X depended upon light and electron microscopical studies of the cutaneous infiltrate and cells obtained from the cerebrospinal fluid. The recurrent subdural effusions responded well to cranial irradiation. Although juvenile xanthogranuloma is generally a benign cutaneous disorder, a spectrum of naevoid proliferative changes involving systemic organs may occur.

Juvenile xanthogranuloma (JXG) is typically a benign dermatosis which commences in infancy and may extend considerably before eventually undergoing spontaneous and complete resolution. Blank, Eglick & Beerman (1949) described the first case of extracutaneous involvement in a patient who developed ocular lesions of JXG. Since then several cases of extracutaneous JXG have been reported (Webster, Reister & Harman, 1966), the most frequent site being the eye, with few reports of visceral non-ocular lesions. These extracutaneous lesions may occur without associated cutaneous involvement (Saraux *et al.*, 1967).

In our case, the child exhibited very extensive cutaneous involvement with JXG but presented initially with recurrent bilateral subdural effusions. The clinical features, light and electronmicroscopic findings are described and meningeal involvement as a cause of the subdural effusion is discussed.

HISTORY

The patient, a girl, was born by forceps delivery at 39 weeks after a normal pregnancy. At 2 months of age she was noticed to have 'freckles' on her face which her mother describes as flat brownish-yellow spots. At 5 months, her mother noticed that she seemed 'top heavy', and was unable to sit properly

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and at 7 months she presented to the local hospital with increasing head size. Bilateral subdural effusions were diagnosed by a computerized tomographic scan, but attempts at drainage by a subdural-pleural shunt were unsuccessful. At this time, the rash on the face and trunk had become more florid presenting as an eruption of yellowish macules and papules on an erythematous background (Fig. 1). These became confluent and plaque-like over the forehead and scalp. Individual yellow papules were scattered over the trunk. A diagnosis of histiocytosis X was considered clinically to be likely. The diagnosis of JXG was established by histopathological and ultrastructural studies.

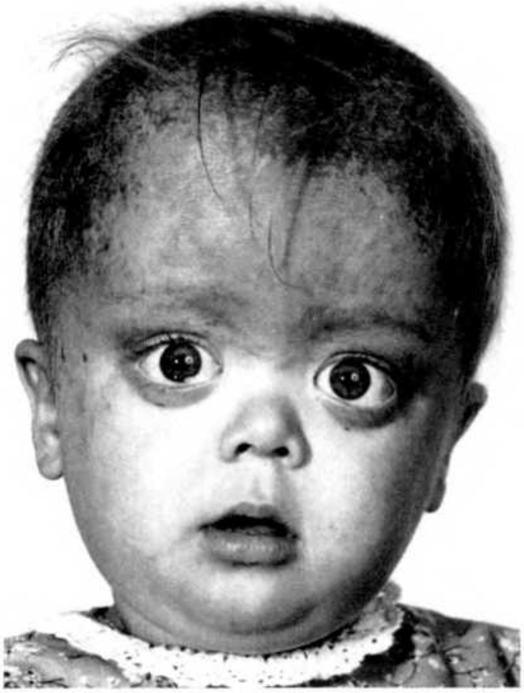

FIGURE 1. Juvenile xanthogranuloma. Yellow-brown maculopapular semi-confluent eruption on scalp and forehead. Skull outline and proptosis are evidence of hydrocephalus.

At 14 months the subdural effusions required repeated tapping and on several occasions cytologically normal histiocytes were found in cytocentrifuged specimens of cerebrospinal fluid. As the process involving the dura appeared to be evolving into a more aggressive phase, cranial irradiation to a total of 2,875 rads over 3 weeks was carried out. Chemotherapy with 6 weekly courses of vincristine, cyclophosphamide and prednisone was instituted following radiotherapy.

In the 12 months subsequent to radiotherapy, the subdural effusions have required tapping on only one occasion and her development progressed normally. The skin eruption however was not improved by radiotherapy or chemotherapy. There has been no objective developmental retardation and no focal cerebral signs. Chemotherapy was stopped after 6 months because of an opportunistic chest infection.

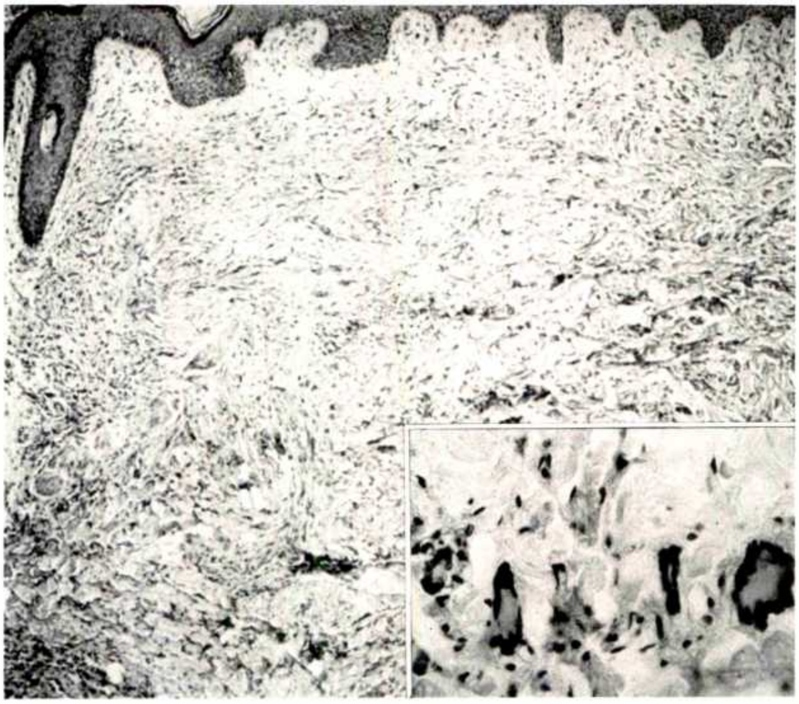

FIGURE 2. Juvenile xanthogranuloma (H & E, × 26). A polymorphous cellular infiltrate comprising histiocytes, fibroblasts and giant cells occupies the dermis. (inset) Juvenile xanthogranuloma (H & E, × 256). Numerous Touton giant cells are present in the dermal infiltrate.

Investigations

Chest X-ray normal; liver/spleen scan—liver and spleen both appeared diffusely enlarged with no focal defects within them; bone scan—large skull, no evidence of bony disease; bone marrow—normal; CSF—cytologically normal histiocytes were present on several occasions.

HISTOPATHOLOGY

Biopsy material from individual papules and the more confluent eruption showed similar changes. The epidermis was normal showing no spongiosis or infiltration by mononuclear cells. In the papillary and reticular dermis there was a polymorphous infiltrate of fibroblasts and mononuclear cells comprising predominantly normal histiocytes, some of which possessed foamy cytoplasm (Fig. 2). Lymphocytes, eosinophils and giant cells, many of Touton type, were also present (Fig. 2, inset). The appearances were those of juvenile xanthogranuloma.

Electron microscopy

In the dermis the findings on light microscopy were confirmed. Numerous active histiocytes many of which contained fat droplets without limiting membranes, dense bodies, myeloid bodies and occasional coated vesicles were present (Fig. 3). No cells containing Langerhans granules were found in the dermis.

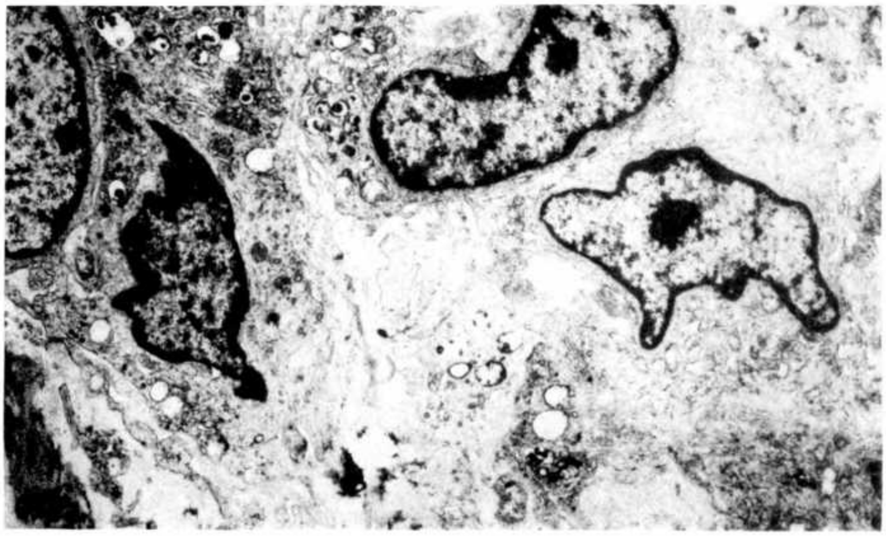

FIGURE 3. Juvenile xanthogranuloma (E.M, × 6,000). Dermal infiltrate comprising normal mature histiocytes containing fat droplets, dense bodies and secondary lysosomes.

DISCUSSION

In this patient the very florid cutaneous eruption of JXG demonstrated the difficulties that can be experienced in differentiating this disease from histiocytosis X. Clinically, the eruption of JXG has a more yellow appearance than that characteristically found in histiocytosis X (Beare & Cunliffe, 1980). JXG typically does not show the purpuric cutaneous lesions commonly seen in the malignant disorders, and diabetes insipidus and lytic bone lesions have never been described. Individual lesions of JXG, however, have been described in patients with histiocytosis X (Altman & Winkelmann, 1963). Histologically, the polymorphism of the infiltrate, absence of abnormal histiocytes, absence of epidermal involvement and the presence of Touton giant cells usually allows fairly simple differentiation from histiocytosis X. Ultrastructurally, the abnormal cells in histiocytosis X of all varieties show Langerhans granules in the cytoplasm (Wolff, 1979) while JXG only shows active histiocytes with none of these specific granules (Esterly, Sahihi & Medenica, 1972; Garcovich & Celleno, 1980). Fat droplets, dense bodies, myeloid bodies and coated vesicles as found in this patient are typical but non-specific features of juvenile xanthogranuloma (Caputo & Gianotti, 1980). No transitional forms between histiocytosis X and JXG have been reported (Guinnepain & Puissant, 1980). This, in addition to the differences in clinical behaviour and cytology, suggests that the two diseases do not share a common pathogenesis.

Visceral non-ocular lesions of JXG are uncommon but have been reported in a number of different sites. Retroperitoneal, colonic, renal, hepatic, ovarian, testicular, pulmonary and pericardial lesions have been reported (Webster *et al.*, 1966; Guinnepain & Puissant, 1980). Although meningeal biopsy was never performed in our patient, the presence of normal histiocytes in repeated CSF specimens and the response to radiotherapy suggest that the meninges were involved by JXG. Central nervous system involvement has been previously reported by Rush (1979) who described a patient with JXG affecting the third ventricle.

JXG is considered to be a benign disease but should perhaps be compared with the situation in mast cell disease, where a spectrum exists from benign urticaria pigmentosa to potentially fatal systemic mastocytosis. The possibility of systemic involvement should be considered in all patients with JXG and should the involvement cause complications, treatment with radiotherapy has been shown to be effective (Nodl, 1959) as in this case.

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Orf and atopic dermatitis

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SUMMARY

An atypical case of orf (granuloma pyogenicum type with multiple satellite lesions) occurred eruptively in a 16-month-old child with generalized atopic dermatitis.

CASE REPORT

P.E. a 16-month-old girl whose parents are city dwellers, presented with atopic dermatitis from the age of 2 months. The eruption had become almost generalized for the previous several months. Pruritus was intense and the trunk and limbs were covered with a gauze garment so that the child could scratch only the face and the extremities. The child was examined by us for the first time because of a large red nodule under the chin on the right side; it was more or less erosive and oozing, suggesting granulation tissue, but lacking a collar and peripheral groove. The lesion was the size of a large hazel nut and developed very rapidly within 10 days. At the periphery of this lesion, multiple papulo-nodules appeared eruptively within 48 h; their size was smaller than the major lesion and more

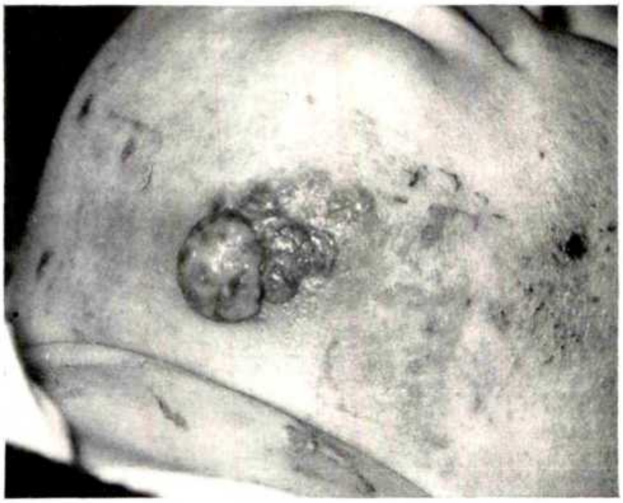

FIGURE 1. Large red nodule under the chin. At the periphery are multiple papulo-nodules.

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uniform, from that of a large lentil to a small pea, with the same erosive nature as the first lesion (Fig. 1). Similar lesions were also found on the face, the wrists and the backs of the hands. The child was feverish (but she also had a bad cold) and the cervical and sub-maxillary lymph nodes were enlarged. The first lesion, examined before us by two different dermatologists, led to the diagnosis of either granuloma pyogenicum or bromoderma and was being treated with local corticosteroids. The child was examined again two days after our first consultation. The appearance was practically identical, but certain lesions were tending to show central umbilication and, on the pseudo-granuloma, several small depressions were observed, suggesting a viral infection. At this time, the parents reported that three weeks earlier the child had entered her uncle's stable in which there were lambs and ewes infected with contagious ecthyma. A diagnosis of orf then became obvious and was confirmed by electron microscopy of infected cells from the primary and secondary lesions. Oral tetracycline and a topical antibiotic were prescribed to prevent secondary infection. The lesions had regressed considerably after 8 days and had practically disappeared after 2 weeks.

DISCUSSION

Whereas the predisposition of atopic dermatitis to superinfection by certain virus diseases (herpes simplex, vaccinia, molluscum contagiosum, verruca) is well known, we have not been able to find any observation in the literature concerning superinfection by orf virus (pox-virus causing contagious ovine ecthyma). The mechanisms of such viral superinfections remain unknown. Most authors attribute the predisposition to a defect of cell mediated immunity and admit the role of excoriations and local corticotherapy in their propagation.

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Occult Bowen's disease in keratinous cysts

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SUMMARY

Bowen's disease (intra-epidermal squamous cell carcinoma) was found in the walls of two cysts excised from the forehead of an 81-year-old man. The inapparent nature of the malignant change underscores the need for routine histological study of rapidly growing cysts.

Bowen's disease can at times humble even the proudest clinician. It may present as a pterygium-like lesion of the conjunctiva (Awan, 1978), as a haemorrhoidal tag (Strauss & Fazio, 1979), as a fellow traveller with a true verruca (Grussendorf & Gahlen, 1974), or as a simple crusted sore of the forearm (Hugo & Conway, 1967). Although Bowen's disease may look clinically innocent, it is a slowly maturing squamous cell carcinoma with the ability to metastasize in the lymphatics in as many as 5% of cases (Rickert, Brodkin & Hutter, 1977). The present report exemplifies the need for histological study. In the course of routine care, two simple rapidly growing epidermoid cysts were excised which surprisingly showed their walls to be infiltrated with the malignant cells of Bowen's disease.

CASE REPORT

This 81-year-old white male with a 30-year history of mild intertriginous psoriasis was seen in May 1979 for evaluation of a 1·6 cm raised, yellowish growth of one year's duration on the right forehead. During the course of the 30 years, we had removed and diagnosed histologically four seborrhoeic keratoses, two warts and a keratinous epithelial cyst (epidermoid cyst). Clinically, the presenting lesion was thought to be a squamous cell carcinoma or keratoacanthoma. A histological diagnosis of Bowen's disease was established (Fig. 1). At this time, a second lesion of Bowen's disease, presenting as a cutaneous horn, was removed from the left side of the forehead.

The patient returned four months later for evaluation of a 2 cm nodule which had been rapidly growing during the past 2 months on the right side of the forehead at the site of the Bowen's disease removed previously. The overlying skin was completely normal, but a freely moveable asymptomatic non-inflammatory mass could be felt in the skin. This was excised and found to be an encapsulated lesion containing two epithelial lined cysts (Fig. 2).

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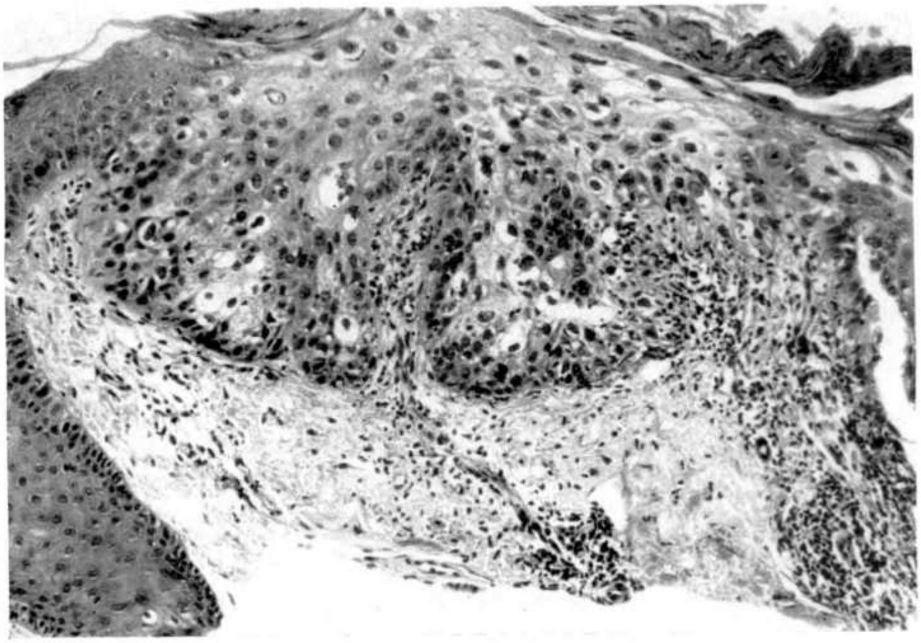

FIGURE 1. Primary papillomatous lesion removed from right side of forehead. Note typical Bowen's disease of the surface epithelium (H & E, × 118).

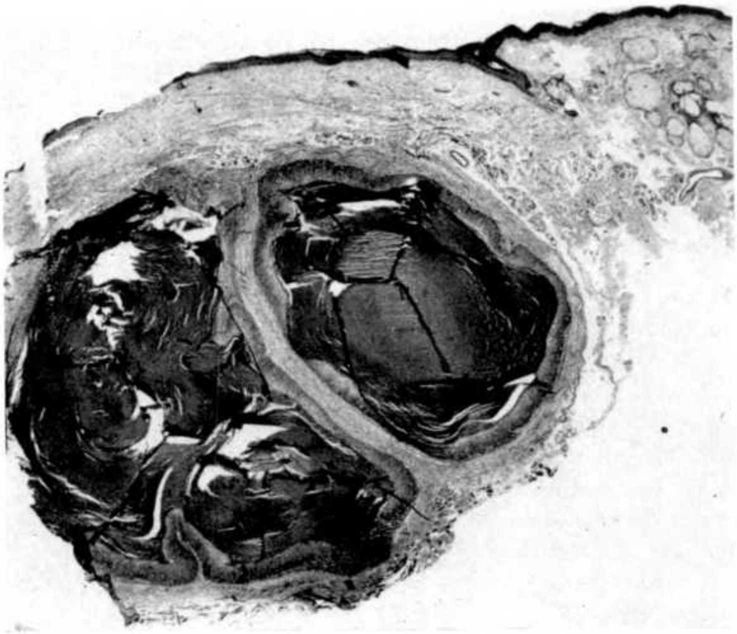

FIGURE 2. Keratin containing cystic structures excised 4 months after removal of primary lesion of Bowen's disease shown in Fig. 1 (H & E, × 13).

Histologically, the epidermis showed an orderly arrangement of normal appearing cells. Scar tissue occupied the dermis overlying the cyst. The cyst was filled with parakeratotic scale and the lining showed changes typical of Bowen's disease. There was a disorderly arrangement of cells and replacement of normal epithelial cells by cells with large hyperchromatic or vesicular nuclei. Vacuolated atypical forms as well as mitotic figures were present (Fig. 3). A diagnosis was made of keratinous epithelial cysts showing Bowen's disease.

Follow up at 6 months revealed no recurrence.

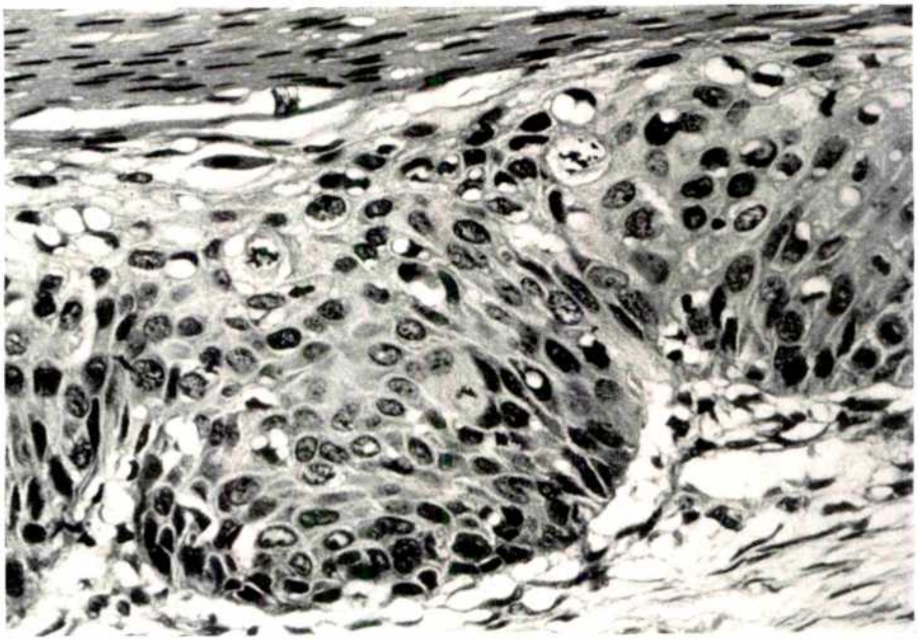

FIGURE 3. Detail of epithelial lining of cyst in Fig. 2. Note diagnostic disorientation of epithelial cells, variation in cell and nuclear size, shape and staining quality, and the mitotic figures (H & E, × 300).

DISCUSSION

Epidermal cysts do not undergo malignant change, although malignant change has been reported in the closely related pilar cyst (Stranc, Bennett & Melmed, 1971; Davidson, Bone & Kiessling, 1976). The present finding of Bowen's disease in the cyst wall is unique and has not been described previously.

The exact origin of the Bowen's malignancy in the wall of the two cysts is not clear. Inasmuch as the patient had had two Bowen's tumours removed previously, one from the same site, there would appear to be a susceptibility to malignant change. One might postulate that malignant cells had been implanted at the time of the original procedure, as with malignant tumours developing along the needle track following a transthoracic biopsy (Sinner & Zajicek, 1976). However, no sutures were employed, haemostasis being achieved by Monsel's solution, and the local xylocaine solution was injected below and peripheral to the lesion.

The tendency of this disease regularly to extend deep down along the outermost sheath of the hair follicle (Graham & Helwig, 1972) is the most likely explanation for the genesis of Bowen's disease in our patient's cysts. This tendency accounts for the high recurrence rate after such treatment as X-ray, curettage and desiccation, cryotherapy or 5-fluorouracil (Coskey, Mehregan & Bryan, 1969; Stevens *et al.*, 1977; Allen, Richardson & Futrell, 1979). In our patient, the curettage may have left residual malignant cell nests in the depths of the hair follicles. These follicular remnants could then have served as progenitors of keratinous cysts. In this circumstance the overlying epidermis would remain free of the disease adding to the clinical mystery of the origin of the malignant keratinous cyst.

This experience again stresses the value of total excision of the lesions of Bowen's disease.

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Urticaria in association with etiocholanolone fever

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SUMMARY

A 17-year-old girl is reported with a history of recurrent febrile episodes during her menstrual bleeding accompanied by a generalised exanthem. Increased plasma levels of unbound etiocholanolone were noticed during the febrile attacks. Both the fever and the skin eruption could be suppressed by oral contraceptives.

Bondy *et al.* (1958) first described increased levels of circulating unconjugated etiocholanolone as a cause of fever. Conjugated or esterified etiocholanolone by contrast did not produce fever. Etiocholanolone (3α-hydroxy-5β-androstan-17-one) is a physiologically occurring metabolite of testosterone and androstenedione, which are secreted by adrenals, testes and ovaries. Free, unconjugated etiocholanolone is formed in the liver and is found in the blood in very low concentrations. Most of it is bound to glucuronic acid and is excreted in this conjugated form.

Since 1958, several additional cases of etiocholanolone fever have been published (Bondy, Cohn & Gregori, 1965; Cara *et al.*, 1963; Essers, Bleifeld & Kaiser, 1971; Feher & Halmy, 1968; Hunstock, Kuhn & Oertel, 1966; Mamon *et al.*, 1960; Mössner & Husmann, 1973). This disorder can be associated with the following clinical symptoms: elevated ESR, leukocytosis, myalgia, arthralgia, abdominal pain, diarrhoea, and vomiting. We have observed an unusual case of etiocholanolone fever associated with an urticarial exanthem. To our knowledge, this syndrome has not been previously reported.

CASE REPORT

The patient was a 17-year-old girl (C.H.) with an uneventful family history. She had a previous history of measles, rubella, appendectomy, tonsillectomy and a seasonal allergic rhinitis. Menarche was at the age of 13, and her menstruation was irregular. Several years ago, she started to suffer

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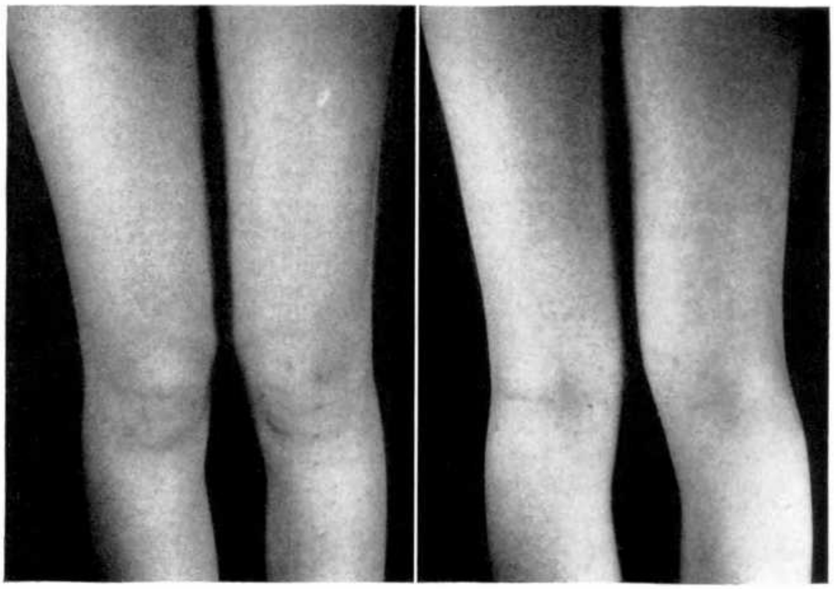
from headache, vomiting, and fever during the first days of the menstrual bleeding. These symptoms persisted for several years, and were accompanied latterly by a supervening generalized urticarial eruption lasting for 3–5 days (Figs 1 & 2). Highly characteristic was the rapidly changing appearance of the eruption, the single lesions lasting for only a few hours. Sometimes the eruption started with a widespread macular non-itching exanthem which transformed within hours to a few large itching urticarial lesions. On other occasions only urticarial lesions were noticed. The episodes lasted for about 3–5 days leaving no permanent changes or purpura. The rash and the other clinical features had some resemblance to vasculitis, but histological examination could not be performed, because the patient did not agree to biopsy.

The temperature during the episodes of fever was 39–41°C.



FIGURES 1 and 2. Widespread macular eruption on the legs in the course of an episode of etiocholanolone fever.

Laboratory findings

WBC 11,200/mm^3 (metamyelocytes 2, band forms 31, polymorphonuclears 58, monocytes 5, and lymphocytes 4%); RBC $3{\cdot}75 \times 10^6$/mm^3, Hb 10·1 g/dl, Fe 55 μg/dl; Na, K, Ca, glucose, creatinine, urea, transaminase, were within normal range. Antistreptolysin titre, C-reactive protein, rheumatoid factor and antinuclear antibodies were negative. Total serum protein, electrophoresis, and immunoelectrophoresis yielded normal results. The C1-esterase inhibitor was within normal limits (24 mg/dl). Bacteriological examinations of the blood, urine and faeces did not reveal any abnormalities. Hormone assays in the serum: Testosterone, estradiol and FSH (4·3 mU/ml) normal, LH (2·7 mU/ml) slightly depressed. Etiocholanolone assay was performed as described by Graef (1971). The results are summarized in Table 1.

The medical, gynaecological, oto-rhino-laryngological and dental examinations were normal.

TABLE 1. Free etiocholanolone plasma levels of the patient (normal range: 0·05–0·45 μg/dl)

During free interval (April 1979)	0·18 μg/dl
During fever attack (April 1979)	1·32 μg/dl
Relapse during prednisolone therapy (10 mg daily) premenstrual (May 1979)	4·38 μg/dl
Relapse during therapy with Ovoresta M (November 1979)	2·43 μg/dl
Relapse after discontinuation of Lyndiol (June 1980)	1·49 μg/dl

Treatment and course

With high-dose glucocorticosteroid therapy (100 mg prednisolone daily), the fever and rash could be suppressed within several hours of administration. A low-dose maintenance therapy (10 mg prednisolone daily) failed to suppress the attacks of fever and skin lesions. In contrast, a dramatic therapeutic effect was obtained by the administration of oral contraceptives: Ovoresta M (0·75 mg lynestrenol, 0·0375 mg ethinylestradiol) and later Lyndiol (2.5 mg lynestrenol, 0·05 mg ethinylestradiol). With this therapy only one relapse was noticed during 18 months.

DISCUSSION

Etiocholanolone is an intermediate of androgen synthesized by the adrenal cortex, ovaries and testes. Unconjugated 5βH-C19-steroids are able to induce fever (Rosendorff & Woolf, 1979). Under normal circumstances, etiocholanolone is conjugated with glucuronic acid by the hepatic enzyme glucuronyltransferase to the corresponding steroid glucuronide. A transient or permanent disturbance of this enzyme might lead to an increased level of unconjugated etiocholanlone and thus initiate fever. The finding of elevated levels of unconjugated etiocholanlone in the blood of the mother of our patient raises the possibility of a genetically determined defect in the conjugation of this steroid in the liver in this disease.

The diagnosis of etiocholanolone fever can be substantiated by the demonstration of increased levels of unconjugated etiocholanolone in the plasma of the patients during the febrile attacks. The etiocholanolone levels return to normal base-line values during the afebrile intervals. Skin lesions such as the urticarial exanthem of our patient have not been described in combination with etiocholanolone fever. The strictly parallel appearance of skin eruptions and the febrile attacks leads to the interpretation that both events are triggered by the increase of the unconjugated etiocholanolone before and during menstruation.

The so called auto-immune progesterone-induced dermatosis (Linse & Hadlich, 1978) is another disease exhibiting skin lesions, including urticaria, in connection with the menstrual cycle (Farah & Shbaklu, 1971; Tromovitch & Heggli, 1967). However, in contrast to the case presented here, the skin lesions appear during the whole luteal phase.

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Correspondence

Treatment of brittle nails and dry eyes

SIR, There is some evidence that patients with defective tear secretion associated with Sjogren's syndrome or the sicca syndrome may respond to treatment with fatty acids, pyridoxine and ascorbic acid (Horrobin & Campbell, 1980). While evaluating this treatment we have found four patients who had brittle nails whose nail and eye conditions improved simultaneously. The patients were treated with the following regime: evening primrose oil (Efamol G), a rich source of essential fatty acids containing both linoleic and gammalinolenic acids, two capsules three times per day; pyridoxine, a vitamin which is required for essential fatty acid metabolism, 25–50 mg per day; ascorbic acid, which enhances conversion of essential fatty acids to prostaglandin E_1, 2–3 g per day (Horrobin & Campbell, 1980).

Patient 1
A 55-year-old woman gave a 6-month history of dry gritty eyes and brittle nails which were prone to split when manicured. On the above regime her eye condition gradually improved and after 1 month she spontaneously reported that her nails had become fully normal.

Patient 2
A 72-year-old woman had an 8-month history of dry, gritty eyes and a 6-month history of her nails splitting and breaking off. Within 3 months of treatment her eyes were moist and she had normal tear secretion. At the same time her nails became normal.

Patient 3
A 53-year-old woman who had a several year history of defective tear secretion had noted that her nails had been brittle and splitting for about 6 months. After 1 month of the above treatment her eyes had improved and her nails had ceased to split.

Patient 4
A 48-year-old woman with rheumatoid arthritis complicated by Sjogren's syndrome stated that her nails were very brittle and her hands very sensitive to exposure to detergents. All therapy was stopped with her consent and she was started on the above regime. After 2 months of treatment, not only was there an increase in tear and saliva production, but for the first time for many years her nails had become hard and could be manicured normally. She also noted a disappearance in skin sensitivity to detergents.

The cause of brittle nails is uncertain and treatment unreliable (Samman, 1978). The incidence of the condition is not known as patients, usually women, rarely complain specifically about it. Our results suggest the need for a carefully controlled trial of this treatment.

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Gartnavel General Hospital, Glasgow — G.C.MCEWAN

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News and Notices

2nd International Symposium on Psoriasis—Israel

The Second International Symposium on the treatment of prosiasis and psoriasis-arthritis, sponsored by the Department of Dermatology, Hadassah University Hospital, Jerusalem and the International Psoriasis Treatment Center, Dead Sea, in collaboration with the Israel Dermatological Society, will be held in Israel, 22–29 November 1981. Further information may be obtained from Mr N.D.Yahalom, General Manager, Health Vacation Center, P.O. B 16581, Tel Aviv 61164, Israel, or from Mrs R.Koppel, Health Vacation Tours, 42 North Audley Street, London W1A 4PY.

Eighth Annual Winter Skin Seminar

The Eighth Annual Winter Skin Seminar will be held at The Given Institute of Pathobiology, Aspen, Colorado, U.S.A., 14–19 February 1982. Further information may be obtained from the Office of Postgraduate Medical Education, The University of Colorado School of Medicine, 4200 East Ninth Avenue, Box C-295, Denver, Colorado 80262, U.S.A.

The Second Zagazig International Conference of Dermatology

The Second Zagazig International Conference of Dermatology will be held in Zagazig University, Egypt, 30 Jan to 2 Feb 1982. It will be co-sponsored by The International Society of Tropical Dermatology and The Society of Physicians of Philadelphia. Abstracts of papers should be submitted before 1 November 1981. Further information may be obtained from The General Secretary, Professor Mohamed Amer, Chairman of Dermatology and Venereology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

West of Scotland Committee for Postgraduate Medical Education and Department of Dermatology, The University of Glasgow. Course on Dermatological Aspects of Basic and Applied Immunology

1 and 2 October 1981

This course is designed both for individuals in training in dermatology and also for those in permanent posts who wish to increase their knowledge of the principles underlying immunological methods which are applied to the diagnosis of dermatological disorders. It will also be of value to non-dermatologists whose work includes clinical dermatology.

Subjects to be covered include humoral immunity-control mechanisms and related disease states, lymphocyte-mediated immunity, the monocyte/macrophage system, immune complex detection, the HLA system, chemotaxis, monoclonal antibodies in dermatology, immunofluorescence and immunoperoxidase techniques, photo-immunology, and the compliment system. Speakers include Professor R.M.Mackie, Professor D.M.V.Parrott, Dr H.M.Dick, Dr P.Friedmann, Dr E.Kirkwood, Dr G.Sandilands, Dr M.L.Turbitt, Dr K.Whaley and Dr P.Wilkinson.

The course will be held in the Anderson College, The University of Glasgow, from 9.15 a.m. to 5 p.m. each day.

Numbers are restricted and early application is advised.

The course fee of £35 includes an informal course dinner on 1 October. Accommodation will be available in a University Hall of Residence at an additional charge.

For further information and application forms, you should contact: The Dean of Postgraduate Medicine, The University of Glasgow, Glasgow G12 8QU.

APPOINTMENTS

Nottingham Area Health Authority

Dr Eric M. Saihan has been appointed as Consultant Dermatologist to Nottingham Area Health Authority (Teaching) with duties at Mansfield and Nottingham, from 1 December 1980.

He graduated in 1968 from Assam and became MRCS (Eng.), LRCP (Lon.) and MRCP in 1974. He worked in the Intensive Care Unit and General Medicine at Newport (Mon.) and later as Medical Registrar at Brighton and Royal Sussex Hospital group. He was Registrar in Dermatology at Bristol Royal Infirmary and later, Senior Registrar in Dermatology at the London Hospital, London.

His main investigative work has been in cyclic nucleotides in psoriasis. He has also published a number of papers of clinical interest.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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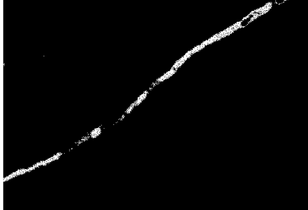

for nurses

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1981. 240 pages, 110 illustrations.
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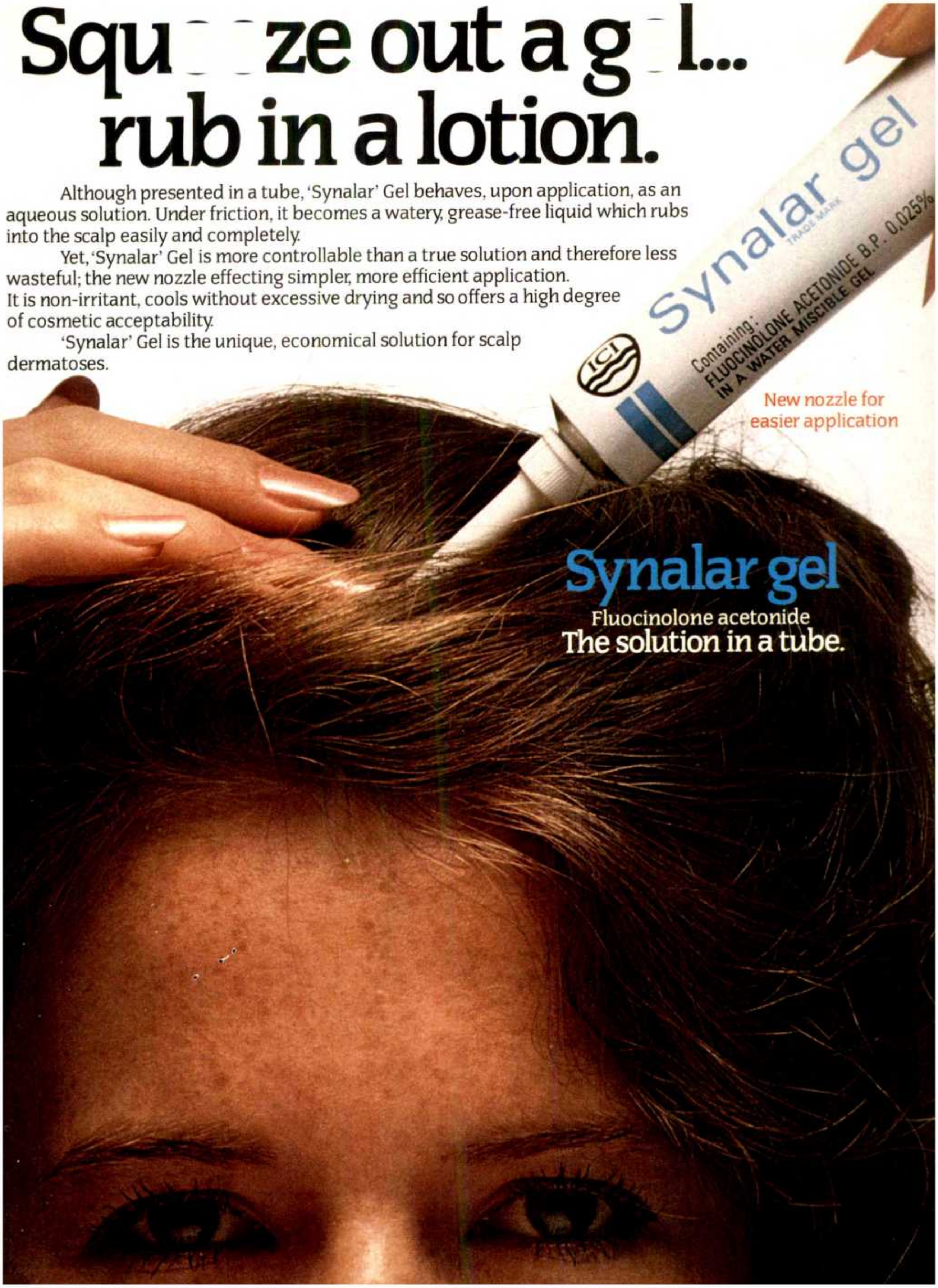

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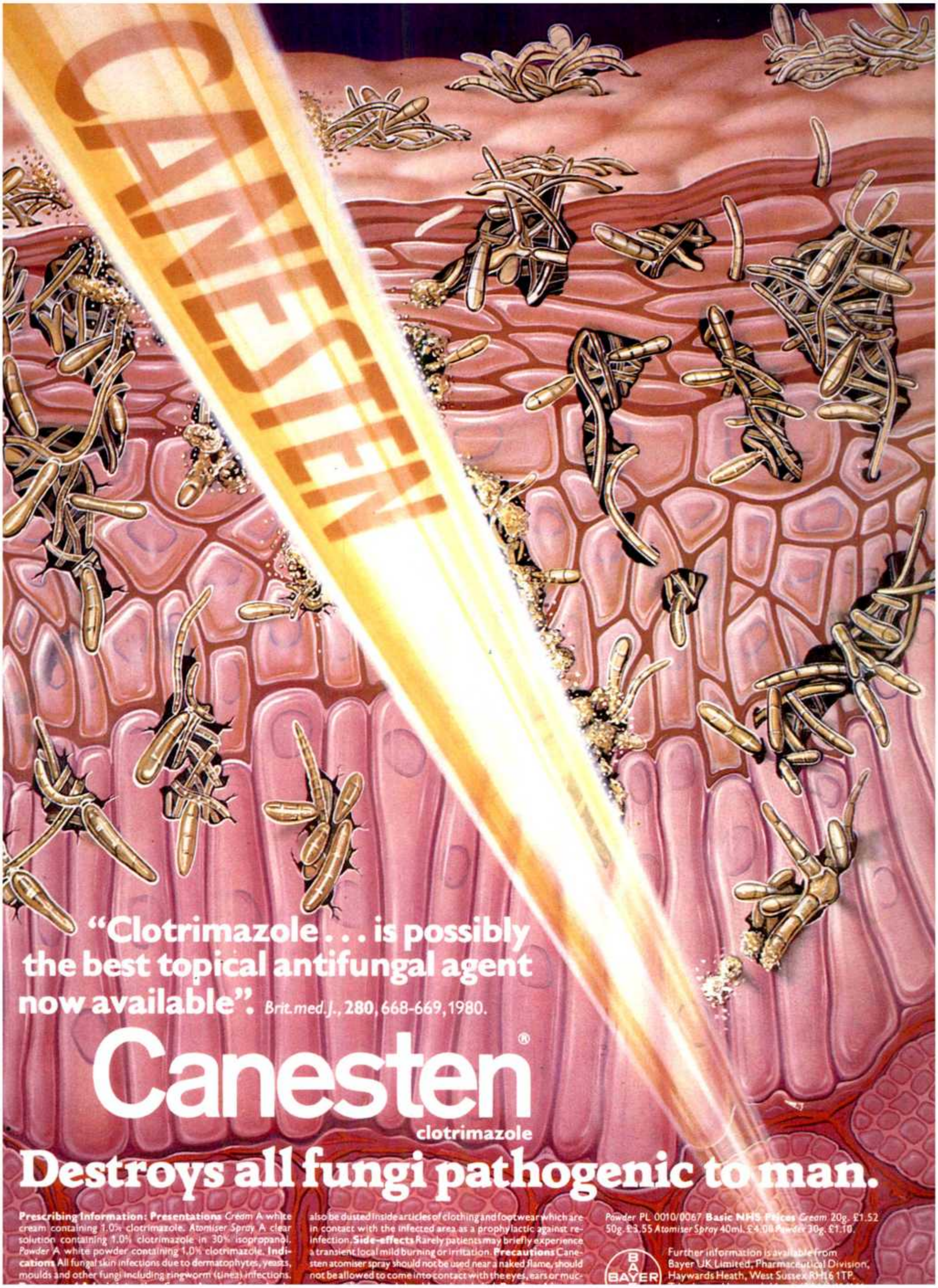
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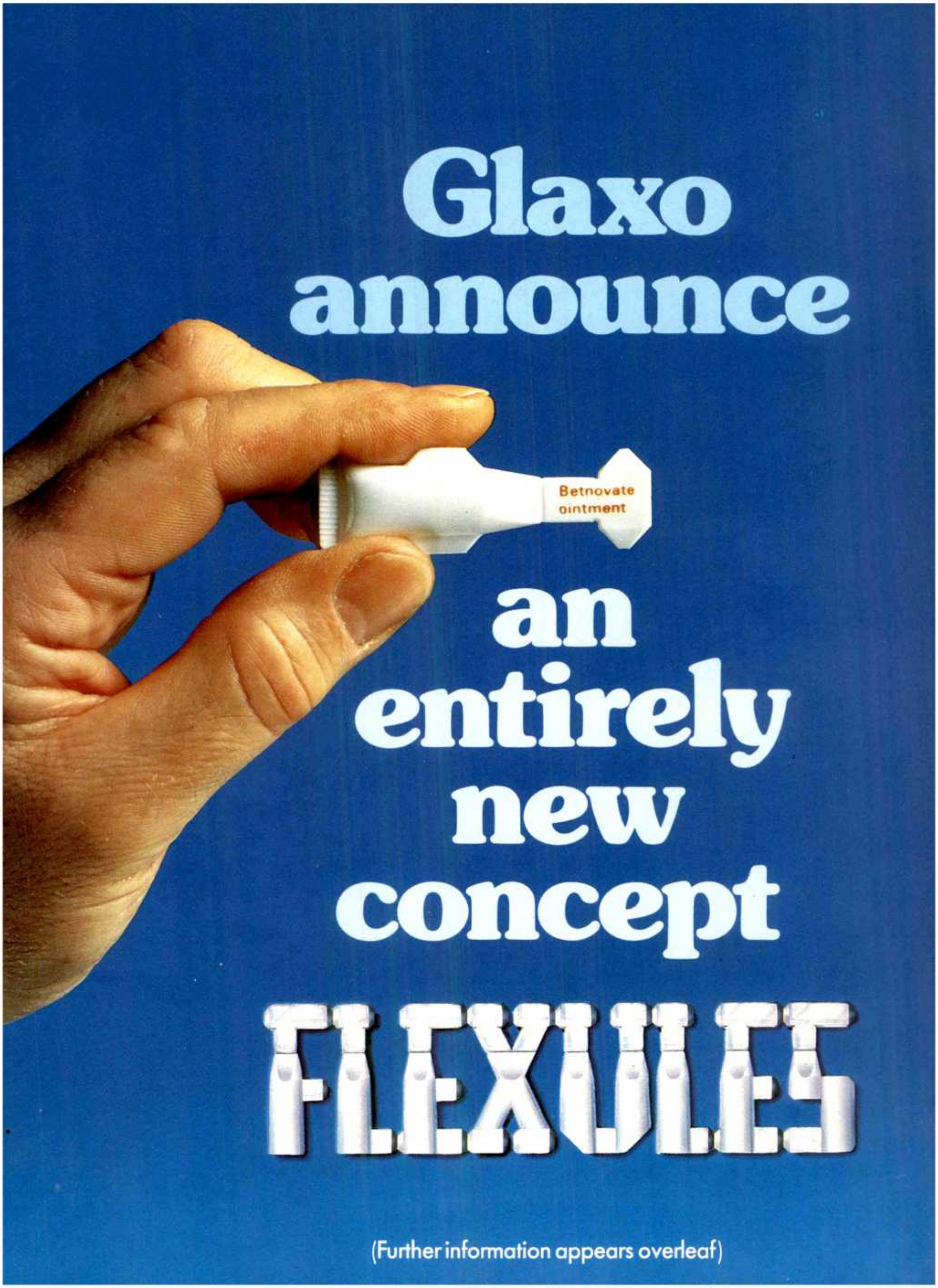

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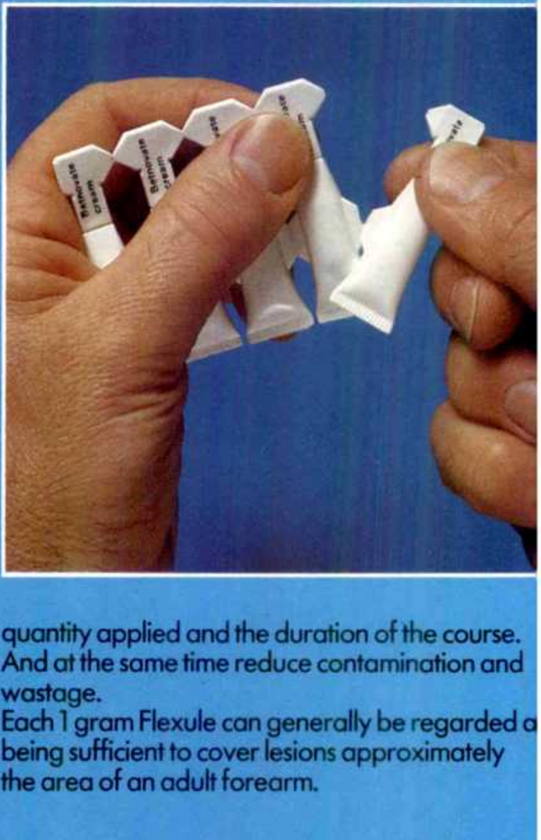

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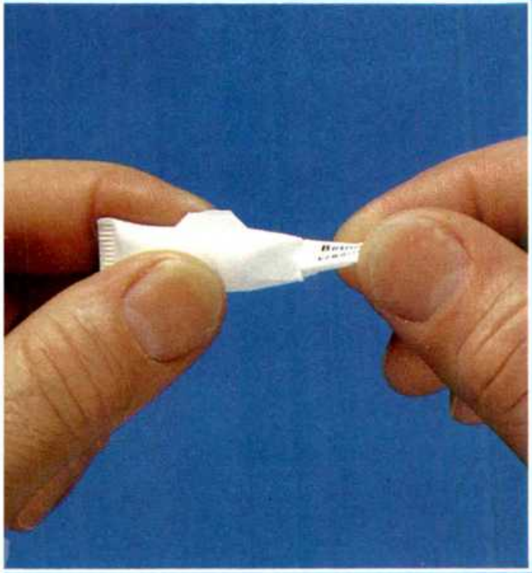

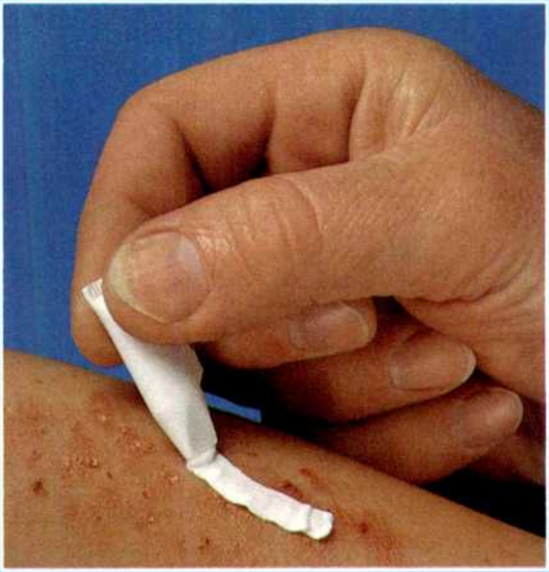

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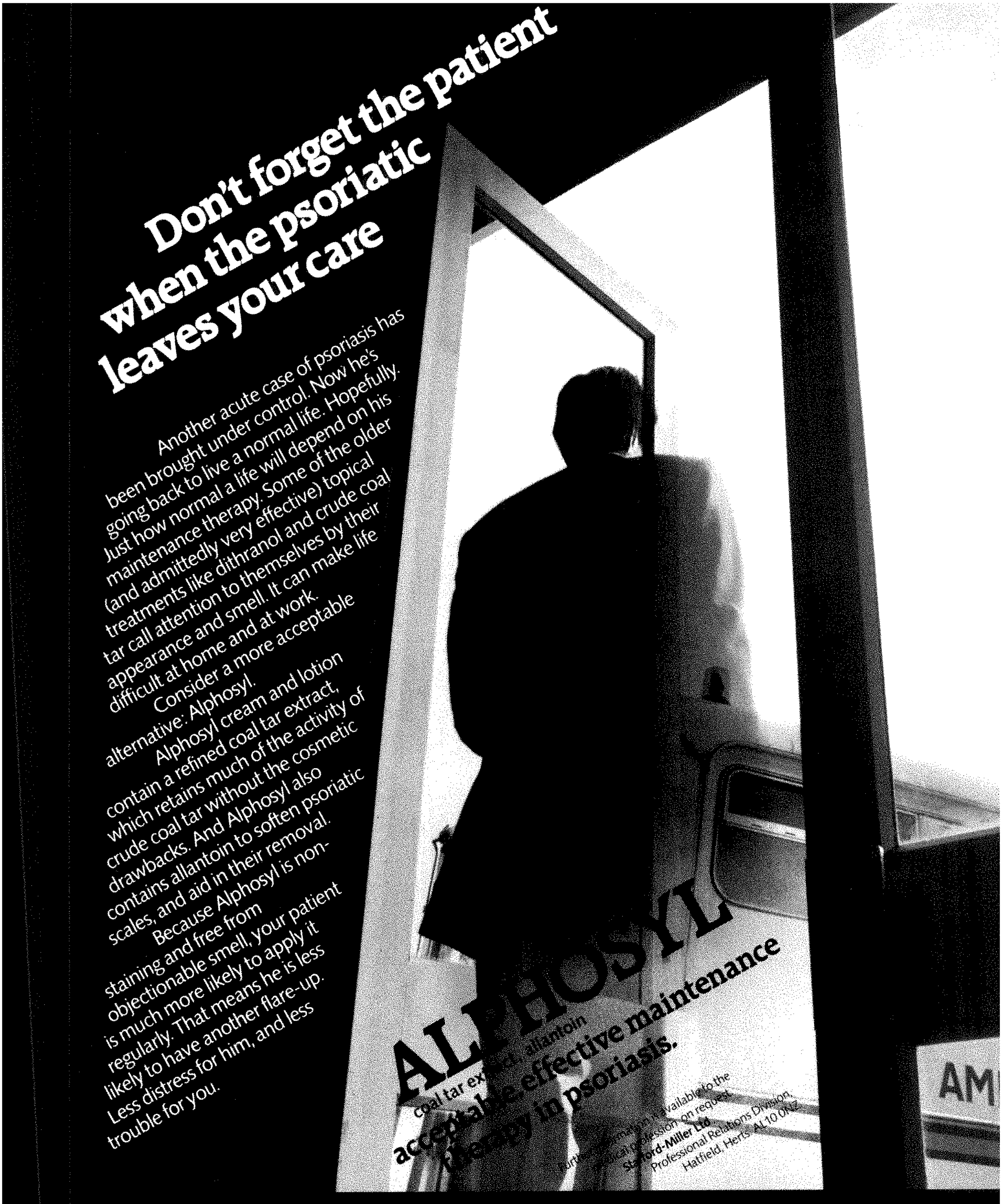

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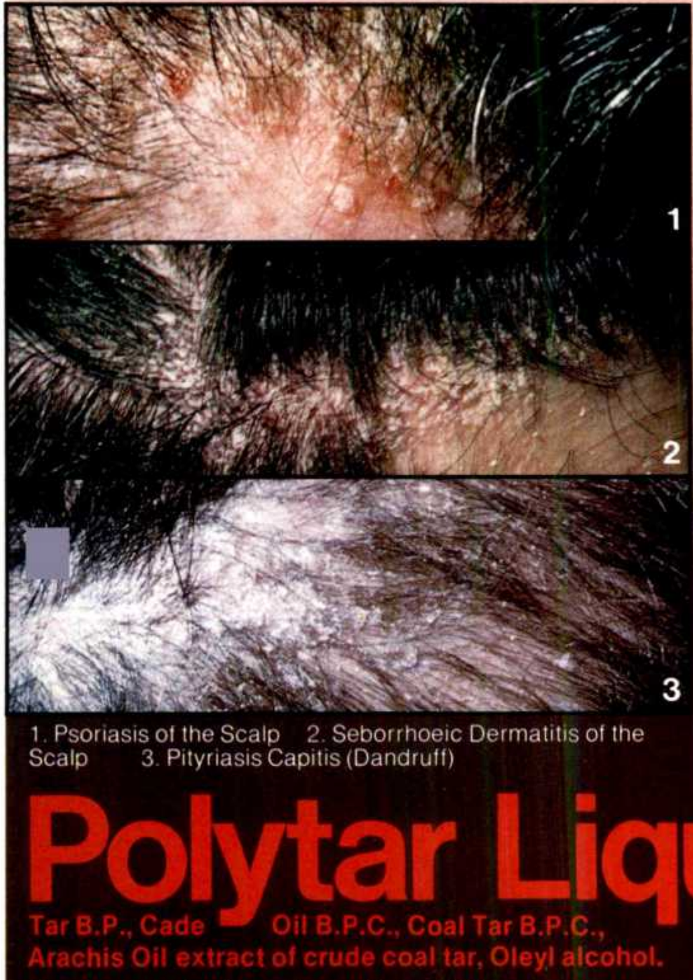

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Contra-indications, warnings, etc. Nil

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British Journal of Dermatology (1981) **105**, 115.

Clinical and Laboratory Investigations

Immunohistochemical demonstration of amyloid P component in skin of normal subjects and patients with cutaneous amyloidosis

S.M.BREATHNACH,* B.BHOGAL,* R.F.DYCK,†‡ F.C. DE BEER,† M.M. BLACK* AND M.B.PEPYS†

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Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road,
London W12 0HS

Accepted for publication 9 February 1981

SUMMARY

The presence of amyloid P component (AP) in dermal deposits of cutaneous amyloidosis was demonstrated by a direct immunofluorescence technique using an antibody to serum amyloid P component (SAP). AP was also shown, for the first time, to be a constituent of normal human skin. It was present at the periphery of dermal elastic tissue fibres, in basement membranes of dermal blood vessels and surrounding eccrine sweat glands but was absent from the dermo-epidermal basement membrane. The staining pattern in cutaneous amyloidosis was morphologically distinctive and readily distinguishable from staining of thickened vascular basement membranes in porphyria. Immunofluorescence with anti-SAP is simple and specific and may become the procedure of choice in the differential diagnosis of amyloidosis.

Amyloid P component (AP) is a glycoprotein which is invariably found in deposits of primary and secondary systemic and endocrine-associated amyloidosis (Cathcart, Skinner & Cohen, 1971; Westermark, Skinner & Cohen, 1975). AP is probably identical to the normal plasma glycoprotein, serum amyloid P component (SAP) (Pepys *et al.*, 1977; Skinner *et al.*, 1980). An immunochemically cross-reactive protein has recently been shown to be a normal constituent of vascular basement membranes (Dyck *et al.*, 1980a,b). We report here that AP is also present in deposits of primary localized cutaneous amyloidosis, and document for the first time the distribution of AP in normal human skin.

---

‡ Present address: University of Saskatchewan, Canada.
Correspondence to M.B.Pepys.

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MATERIALS AND METHODS

Subjects

Ten normal control subjects, fourteen patients with primary localized cutaneous amyloidosis (eight macular, five papular, one nodular), one patient with myeloma-associated amyloidosis and thirty-five patients with unrelated dermatoses were studied (Table 1).

TABLE 1. Immunofluorescence staining of skin biopsy specimens with anti-SAP antibody

Pathological diagnosis	No. of patients studied	Figure
Normal controls	10	1a, b, 2, 3, 4
Primary localized cutaneous amyloidosis		
Macular	8	6
Papular	5	5a, b
Nodular	1	
Myeloma-associated amyloidosis	1	7
Porphyria		
Erythropoietic protoporphyria	3	8
Porphyria cutanea tarda	1	9
Lupus erythematosus	11	
Lichen planus	5	
Bullous pemphigoid	5	
Lichenified Eczema	4	
Miscellaneous		
Leukocytoclastic vasculitis	2	
Papular mucinosis	1	
Erythema multiforme	1	
Psoriasis	1	
Cellular naevus	1	

Antiserum

Monospecific rabbit antiserum to human SAP was raised by immunization of a rabbit with isolated pure SAP (Pepys *et al.*, 1977). This reagent will soon become readily available commercially. The IgG fraction of this serum was digested with pepsin to yield $F(ab')_2$ fragments which were labelled with fluorescein isothiocyanate (FITC, BDH Chemicals, Poole, England) (Goding, 1976). Conjugate eluted from DEAE cellulose and having a ratio of A 495/A 280 <1.0 was used for direct immunofluorescence staining of cutaneous sections (Johnson, Holborow & Dorling, 1978).

Immunohistochemical studies

Cryostat sections 4 μm thick of fresh, unfixed skin biopsy specimens were washed in phosphate buffered saline pH 7·4 (PBS) for 15 min at 21°C. The sections were then stained with optimal dilutions of FITC-$F(ab')_2$ anti-SAP for 30 min at 21°, washed three times with PBS and mounted in buffered glycerol. The immunological specificity of all staining was established by inhibition controls in which the FITC-anti-SAP had previously been absorbed with isolated pure SAP. Fluorescence was read and photographed in the Leitz Orthoplan/Orthomat system on Kodak ASA 400 film. All biopsy specimens were also routinely examined following standard histological staining, and alkaline Congo Red staining and thioflavine T fluorescence where appropriate.

Serum SAP and C-reactive protein levels

These were measured in patients with amyloidosis as previously described (Pepys *et al.*, 1978).

RESULTS

Normal skin

Direct immunofluorescence staining of normal skin with anti-SAP produced bright specific fluorescence associated with dermal elastic tissue fibres which was abolished by prior absorption of the fluor with pure SAP (Fig. 1a, b). Thin superficial oxytalan fibres in the papillary dermis directed perpendicularly to the dermo-epidermal junction stained homogeneously throughout their diameter. Thicker eulanin and elastic fibres in the reticular dermis were seen as tube-like structures in which specific staining was confined to the periphery (Fig. 2). Transverse section of these fibres confirmed the presence of a brightly fluorescent outer rim surrounding a non-fluorescent core. Specific linear fluorescence of basement membranes in the walls of small dermal blood vessels (Fig. 3) and surrounding sweat glands (Fig. 4) was also noted. Non-specific staining of the stratum corneum was present in all sections. Dermo-epidermal basement membrane zone fluorescence was not observed in normal skin.

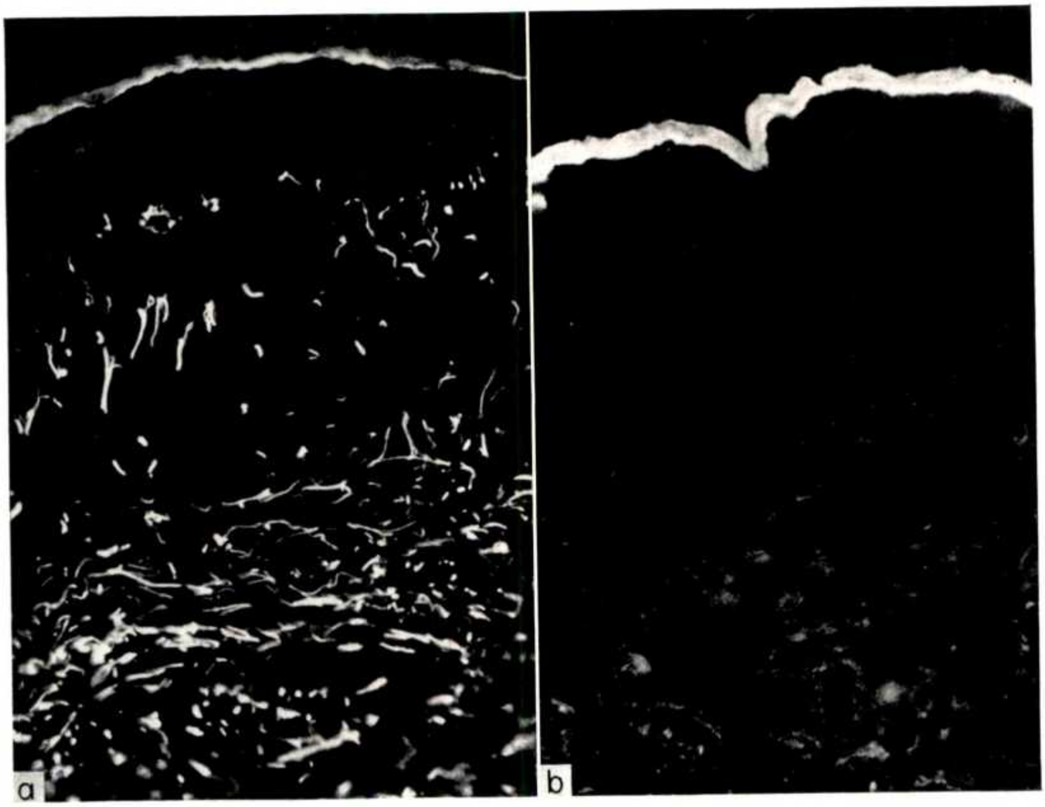

FIGURE 1. Immunofluorescent staining of normal skin with anti-SAP antibodies. Specific fluorescence associated with dermal elastic fibres (a), inhibited completely by preabsorption of the fluor with isolated SAP (b). The stratum corneum shows non-specific uptake of fluor (magnification × 126).

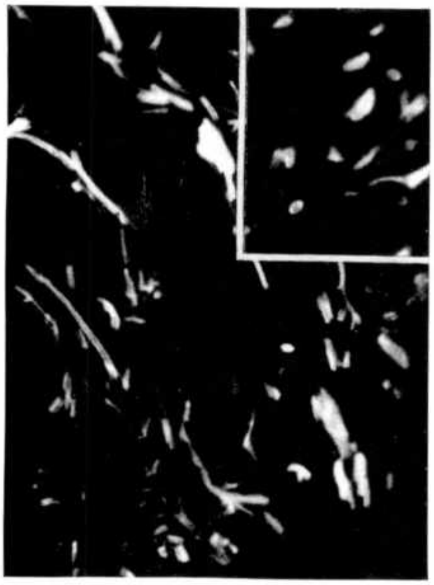

FIGURE 2. Immunofluorescent staining of normal skin with anti-SAP antibodies. The periphery of the elastic fibres is stained as shown by the tubular appearance when viewed both longitudinally and in transverse section (inset) (magnification × 360).

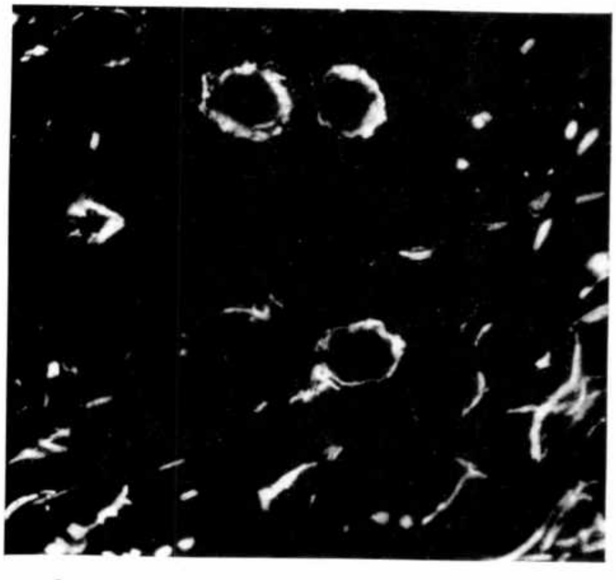

FIGURE 3. Immunofluorescent staining of normal skin with anti-SAP antibodies. Linear fluorescence of basement membranes of small dermal blood vessels (magnification × 360).

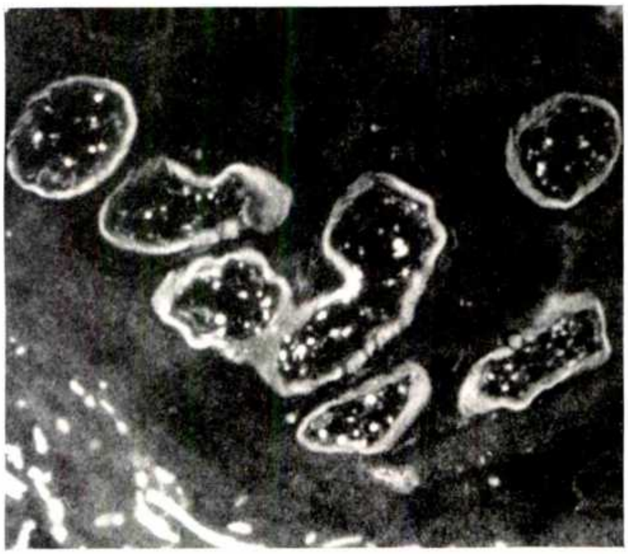

FIGURE 4. Immunofluorescent staining of normal skin with anti-SAP antibodies. Linear fluorescence surrounding eccrine sweat glands. The bright structures within the glands are autofluorescent lipid deposits (magnification × 360).

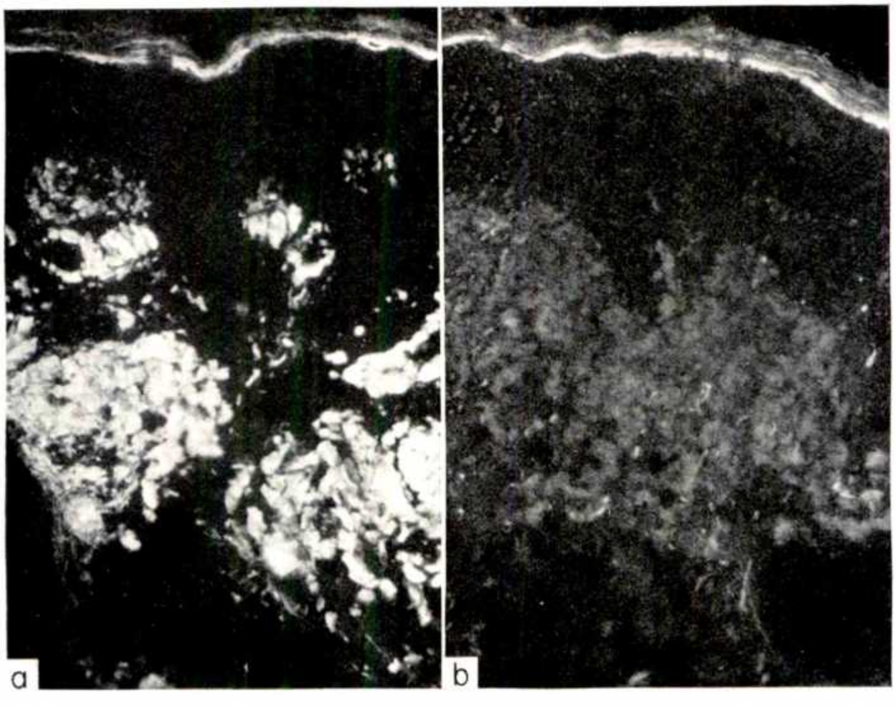

FIGURE 5. Immunofluorescent staining with anti-SAP antibodies of primary localized cutaneous amyloid deposits (papular variant). Large brightly staining masses of amyloid (a) which do not stain after preabsorption of the fluor with isolated SAP (b). There is non-specific fluorescence of the stratum corneum (magnification × 126).

Cutaneous amyloidosis

Morphologically distinctive staining of dermal deposits with anti-SAP was seen only in cases with amyloidosis and was again abolished by prior absorption of the fluor with pure SAP (Fig. 5a, b). There were no false positive or negative results. The staining pattern in all types of primary localized cutaneous amyloidosis was essentially similar and was characterized by the presence of small brightly fluorescent globules in the papillary dermis. In macular amyloidosis the globules remained discrete (Fig. 6), but in the papular and nodular variants there was a tendency towards aggregation with the formation of large masses showing confluent bright fluorescence (Fig. 5a,b). In the case of myeloma-associated amyloidosis there was diffuse specific staining of deposits in the papillary and upper reticular dermis; fluorescence was, however, relatively faint and noticeably weaker than that of dermal elastic tissue fibres (Fig. 7).

Porphyria

Bright specific continuous linear fluorescence of greatly thickened basement membranes of blood vessels in the papillary and upper reticular dermis with anti-SAP was observed in erythropoietic protoporphyria (EPP) (Fig. 8). Similar but less extensive changes were seen in papillary blood vessels in porphyria cutanea tarda (PCT). Linear fluorescence along the dermo-epidermal junction in EPP was caused by a local proliferation of oxytalan fibres in this region. There was disruption of the normal staining pattern of elastic tissue in the upper dermis in the case of PCT (Fig. 9). Areas of bright fluorescence with an irregular outline were composed of accumulations of individual thickened and distorted eulanin and elastic fibres.

Lupus erythematosus, lichen planus and erythema multiforme

Colloid bodies in the upper dermis did not fluoresce with anti-SAP. Some biopsy specimens from patients with lupus erythematosus showed abnormalities of staining of dermal elastic tissue resembling those found in PCT.

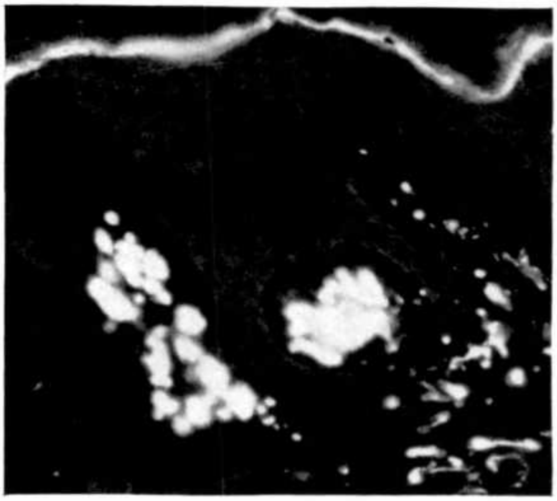

FIGURE 6. Immunofluorescent staining with anti-SAP antibodies of primary localized cutaneous amyloid deposits (macular variant). Discrete globules within the papillary dermis are brightly stained (magnification × 360).

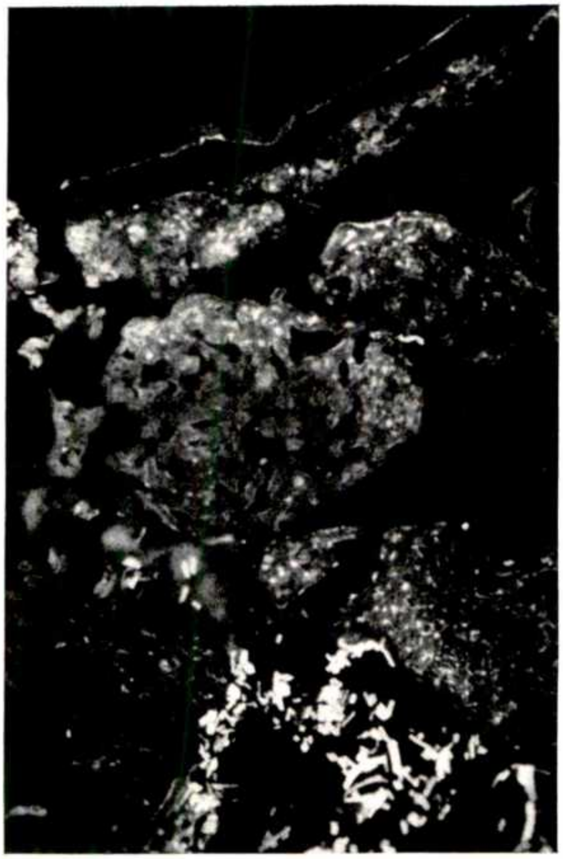

FIGURE 7. Immunofluorescent staining with anti-SAP antibodies of dermal deposits of myeloma-associated amyloid. Diffuse specific staining of extensive amyloid deposits which is weaker than the staining of adjacent elastic fibres (magnification × 126).

Other conditions

No significant abnormalities in the pattern of staining with anti-SAP were demonstrated in bullous pemphigoid, lichenified eczema, leukocytoclastic vasculitis, papular mucinosis, psoriasis or cellular naevus.

Serum SAP and CRP levels

Serum levels of SAP and CRP were measured in thirteen of the fourteen patients with primary localized cutaneous amyloidosis and in all cases fell within the normal range. The one patient with myeloma-associated amyloid had a raised CRP concentration (41 μg/ml).

DISCUSSION

Amyloid P component (AP) has recently been identified as a normal constituent of vascular basement membranes (Dyck *et al.*, 1980a, b). Our results show that AP is present in the basement membranes

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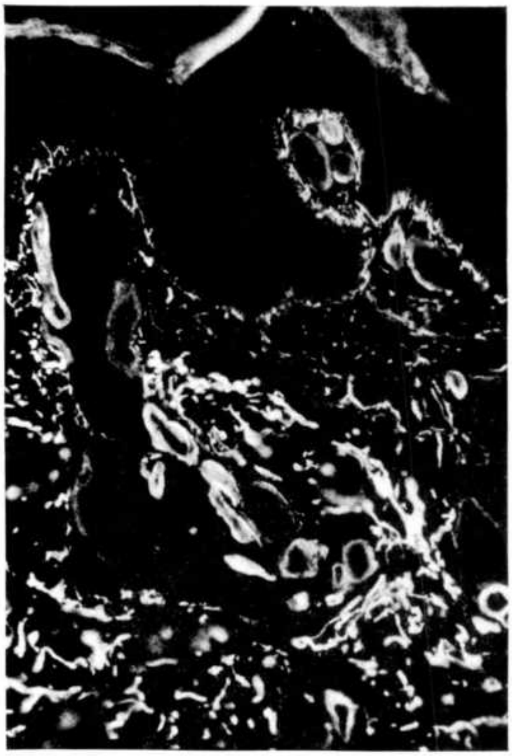

FIGURE 8

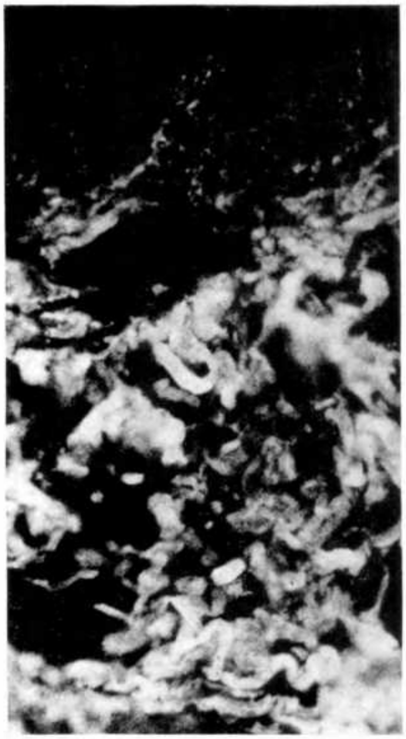

FIGURE 9

FIGURE 8. Immunofluorescent staining with anti-SAP antibodies of skin from a patient with erythropoietic protoporphyria. Specific linear fluorescence of thickened vascular basement membranes in the dermis (magnification × 126).

FIGURE 9. Immunofluorescent staining with anti-SAP antibodies of skin from a patient with porphyria cutanea tarda. Specific staining of thickened and distorted elastic fibres in the upper dermis (magnification × 360).

of small dermal blood vessels and surrounding eccrine sweat glands, but is absent from the dermo-epidermal basement membrane. Furthermore, we have demonstrated that AP is a normal structural component of dermal elastic tissue. Elastic tissue in the dermis consists of accumulations of three different types of fibre which are thought to correspond to consecutive stages in elastogenesis (Fahrenbach, Sandberg & Cleary, 1966; Greenlee, Ross & Hartman, 1966; Cotta-Pereira, Guerra Rodrigo & Bittencourt-Sampaio, 1976). Thin superficial oxytalan fibres in the papillary dermis are formed of bundles of tubular microfibrils each measuring approximately 10 nm in diameter. Thicker eulanin and mature elastic fibres in the reticular dermis are composed of a central amorphous elastin core surrounded by an envelope of tubular microfibrils. Immunofluorescence with anti-SAP produced homogeneous staining of oxytalan fibres, whilst fluorescence in eulanin and elastic fibres was confined to the periphery. Using immunoelectron microscopy AP has been demonstrated in the same site as the microfibrillar protein (Breathnach *et al.*, 1981). The presence of AP as an integral constituent of normal tissue structures as well as of amyloid deposits is of considerable pathophysiological interest and may be of importance in the pathogenesis of amyloidosis.

Immunofluorescence with anti-SAP revealed that AP is present in dermal deposits of all types of cutaneous amyloidosis. The pattern of staining in amyloidosis was morphologically quite distinctive and was of diagnostic value. The intensity of fluorescence in the case of myeloma-associated amyloidosis was relatively faint, suggesting that there was a reduced concentration ratio of AP to amyloid fibril material compared with primary localized cutaneous amyloidosis. Continuous linear staining of greatly thickened basement membranes of upper dermal blood vessels with anti-SAP in porphyria, readily distinguishable from the pattern of fluorescence observed in amyloidosis, was striking. The characteristic reduplication of the vascular basal lamina seen with the electron microscope in porphyria (Ryan & Madill, 1968; Epstein, Tuffanelli & Epstein, 1973) therefore involves increased deposition of AP in addition to type IV collagen and laminin (Wick, Honigsmann & Timpl, 1979). The abnormality of staining of dermal elastic fibres in the case of porphyria cutanea tarda and in some patients with lupus erythematosus was also different from the fluorescence pattern in amyloidosis and probably represents a form of elastosis (Epstein *et al.*, 1973; Schmitt, Thivolet & Perrot, 1972).

Recent immunohistochemical studies of amyloid by Fujihara *et al.* (1980) demonstrated clearly that AL-type fibrils bind only anti-idiotypic antibodies raised by immunization with the patient's own amyloid fibrils. Although anti-AA antibodies, which are difficult to raise and obtain in pure form, bind to all fibrils of AA type there is as yet no evidence that this protein is present in deposits of primary localized cutaneous amyloid. Indeed preliminary tests for potassium permanganate sensitivity of congophilia (Wright, Calkins & Humphrey, 1977) in this condition indicate that, unlike AA fibrils, these fibrils are resistant (unpublished observations). We therefore conclude that immunhistochemical staining with anti-SAP antibodies, which is both simple and specific, may be a valuable method for the diagnosis of cutaneous amyloidosis and its differentiation from other cutaneous hyalinoses.

ACKNOWLEDGMENTS

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Establishment of a cell line of human skin squamous cell carcinoma *in vitro*

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SUMMARY

A cell line of a squamous cell carcinoma of human skin (HSC-1) has been established *in vitro*, and successfully maintained proliferative in continuous tissue culture for about 2½ years since March 1978. Another cell line (HSC-1b) has been established *in vitro* from the recurrence of the same cancer. It has been maintained for about 8 months since December, 1979. 3T3 feeders were used in the initial cultures in both cases. The cells grow in a monolayer *in vitro* and are epithelioid with anaplastic features. Chromosome analysis of HSC-1, frozen and thawed HSC-1, the transplanted tumour in a nude mouse and HSC-1b all showed hypotetraploidy with the modal number 80, which reconfirms that HSC-1 derives from the original skin tumour.

The cell line is available to other investigators.

Cell lines of human squamous cell carcinomas have been established from the cervix (Moore, Sabachewsky & Toolan, 1955; Atkin & Baker, 1966; Ogawa, 1967; Friedel *et al.*, 1970), mouth (Moore *et al.*, 1955; Eagle, 1955; Syverton & McLaren, 1957), larynx (Moore *et al.*, 1955), oesophagus (Syverton & McLaren, 1957), urinary bladder (O'Toole *et al.*, 1976) and lung (Tsuji *et al.*, 1972). However, there have been only few reports on the establishment of cell lines of squamous cell carcinoma from human skin (Moore *et al.*, 1975; Sinkovics *et al.*, 1978). Since human skin carcinomas are easily observed, diagnosed and treated, with a relatively good prognosis compared with other internal tumours, they may be good material for the investigation of tumours, especially to study the relationship between tumour and host, immunologically, biologically and therapeutically.

As a first step in studies of skin cancer, we have tried to establish a cell system *in vitro*. Here we report our success in the establishment of cell lines (designated as HSC-1 and HSC-1b) derived from a case of human skin squamous cell carcinoma.

MATERIALS AND METHODS

Materials

Dulbecco modified essential medium with 4·5 g/l glucose (DMEM), minimum essential medium

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with Hank's salts (HMEM) and fetal calf serum (FCS) were purchased from Osaka Dainihon Chemical Company. Plastic culture dishes are of Nunclon (A/S NUNC, Denmark). 3T3 cells derived from Swiss Albino were purchased from Osaka Dainihon Chemical Company. Nude mice (BALB/c) were purchased from Japan Clea, Tokyo.

Methods

Establishment of HSC-1.

(1) Primary culture: an ulcerated tumour on the back of the right hand of a 75-year-old Japanese man was surgically excised and minced into about 1 mm^3 portions on 2 March 1978, which were placed in 3·5 cm plastic dishes and cultured in DMEM supplemented with 20% FCS, penicillin (100 units/ml) and streptomycin (50 μg/ml), in a gas phase of 95% air and 5% CO_2. In about 5 days, cells with an epithelial pattern appeared from the tumour material. Fibroblasts surrounded the outgrowth completely in about 14 days. The culture was then exposed to 0·02% EDTA in phosphate buffered saline (PBS) at pH 7·4 for 15 s and washed by blowing PBS through a pipette to remove the fibroblasts. After removing more than 90% of the fibroblasts from the culture, 3T3 cells irradiated with a dose of 4,000–6,000 rad of X-ray (150 V, 1·0 mm Al, 35 cm) were added at the rate of 1×10^5 cells/3·5 cm dish as a feeder layer for the epithelioid cell outgrowth. The same procedure was repeated twice until the first subculture was performed.

(2) Subculture: the first subculture was performed on the 53rd day of the primary culture when the sheet of epithelioid cells occupied about 2/3 of the whole surface of the culture dish. Treatment with 0·02% EDTA was performed as mentioned in 'primary culture' to remove most of the contaminating fibroblasts, next the culture was exposed to 0·02% EDTA-0·05% trypsin solution (1:1) for about 3 min, and then cells were isolated. Isolated cells were subcultured together with lethally irradiated 3T3 cells in 3·5 cm plastic dishes or glass culture bottles at a ratio of 2×10^4–1×10^5 cells to 1×10^5 3T3 cells. When colonies of epithelioid cells grew, pushing away 3T3 cells at the periphery and occupying nearly the whole surface of the culture dish, the same procedure was repeated for the subculture. After the 10th subculture, 3T3 cells were omitted from the culture and only tumour cells were cultured. From the 15th subculture media were changed from DMEM to HMEM plus 5 mM HEPES. Cells were subcultured approximately every 2 weeks.

(3) Electron micrography: tumour specimens excised from the patient were fixed in 5% glutaraldehyde and post-fixed in 2% osmium tetroxide for 2 h at 4°C. The specimens were then dehydrated in an ethanol series followed by propylene oxide, and embedded in Epon. As culture cells, the cells of the 25th passage grown on a coverslip were used. The coverslip with cells on the surface was treated as mentioned above, except for 1 h fixation in osmium tetroxide solution. Thus, the sheet of the culture cells embedded flat was sectioned in planes parallel and perpendicular to the cell monolayer.

(4) Chromosome analysis: colcemid (2 μg/ml) was added to the culture and after 3 h the culture cells were isolated by trypsinization and washed in PBS by centrifugation. The washed cells were suspended in about 8 ml hypotonic solution (1 volume of 0·075 M KCl plus 1 volume of 0·9% sodium citrate), incubated at 37°C for 10 min and centrifuged for 5 min at 1,000 r.p.m. The pellet was fixed in a small amount of a fixative composed of equal volumes of acetic acid and methyl alcohol and the fixed cells were dropped onto a glass slide, dried under an electric bulb (100 W), and stained with Giemsa. More than 400 cells in metaphase were chosen under a microscope, photographed, and subjected to chromosome analysis.

(5) Storage of cells at −80°C: some of the cells from the third subculture were suspended in DMEM containing 20% FCS and 10% dimethyl sulphoxide, and stored in a refrigerator at −80°C. After 1½ years, the cells were thawed quickly in water at 37°C. An aliquot of the cells was submitted

to a trypan blue exclusion test to estimate the cell survival. Other cells were cultured again and submitted to chromosome analysis after three passages.

(6) Transplantation experiment on nude mice: the experiments were repeated nearly 10 times, but no tumour growth was observed except for one tumour on one occasion with the cells of the 42nd subculture. In that case, 20 million cells of the 42nd subculture suspended in 0·5 ml PBS were inoculated through a syringe subcutaneously into the back of a nude mouse. After about 20 days, a tumour appeared and started to grow. By 55 days, the mouse was dying with a big tumour on the back, when it was sacrified and autopsied. The excised tumour was submitted to cell culture and histological analyses.

Establishment of HSC-1b. On 2 December 1979, the recurrent tumour on the right hand was excised, minced and cultured as in the first treatment. The outgrowth of the tumour cells grew slowly but steadily with the aid of 3T3 feeder layers, until the first subculture was performed on 22 February 1980, when the cells were isolated by the EDTA-trypsin treatment. The isolated cells were cultured on the 3T3 feeder layers. From the second subculture 3T3 feeders were omitted.

RESULTS

Original tumour

Figure 1 shows the histological findings of the excised tumour. The cells possessed a slightly eosinophilic cytoplasm and few intercellular bridges. They were polygonal or elongated in shape, the majority being atypical with pyknotic nuclei. Horn pearls were rarely found (Fig. 1A). The electron micrograph (Fig. 1B) showed an indented nucleus and many bundles of filaments and desmosomes in the cytoplasm. Histologically, this tumour was relatively undifferentiated.

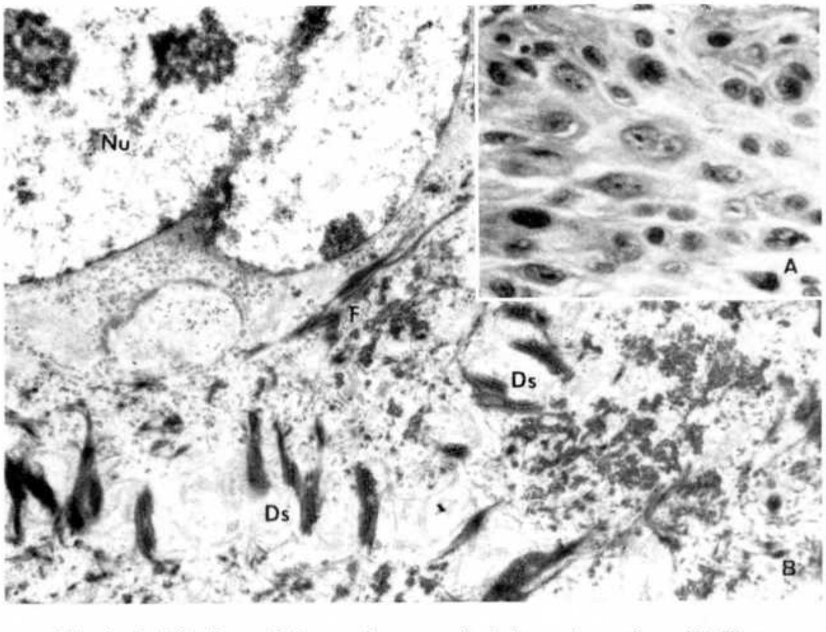

FIGURE 1. Histological findings of the carcinoma excised from the patient. (A) Tumour cells are polygonal or elongated in shape and the majority are atypical with pyknotic nuclei (H & E, × 320). (B) The ultrastructure of the excised tumour (× 9,600). Bundles of tonofilaments (F), desmosomes (Ds) and an indented nucleus (Nu) are seen.

Recurrence of tumour

Several months after the first excision, there was a recurrence of the tumour at the edge of the excision. The recurrent tumour was treated with local injections of bleomycin, grenz rays and excisions; however it repeatedly recurred until amputation of the hand was performed. Recurrent tumours showed findings histologically identical to those of the original tumour, except for some increase in keratinization.

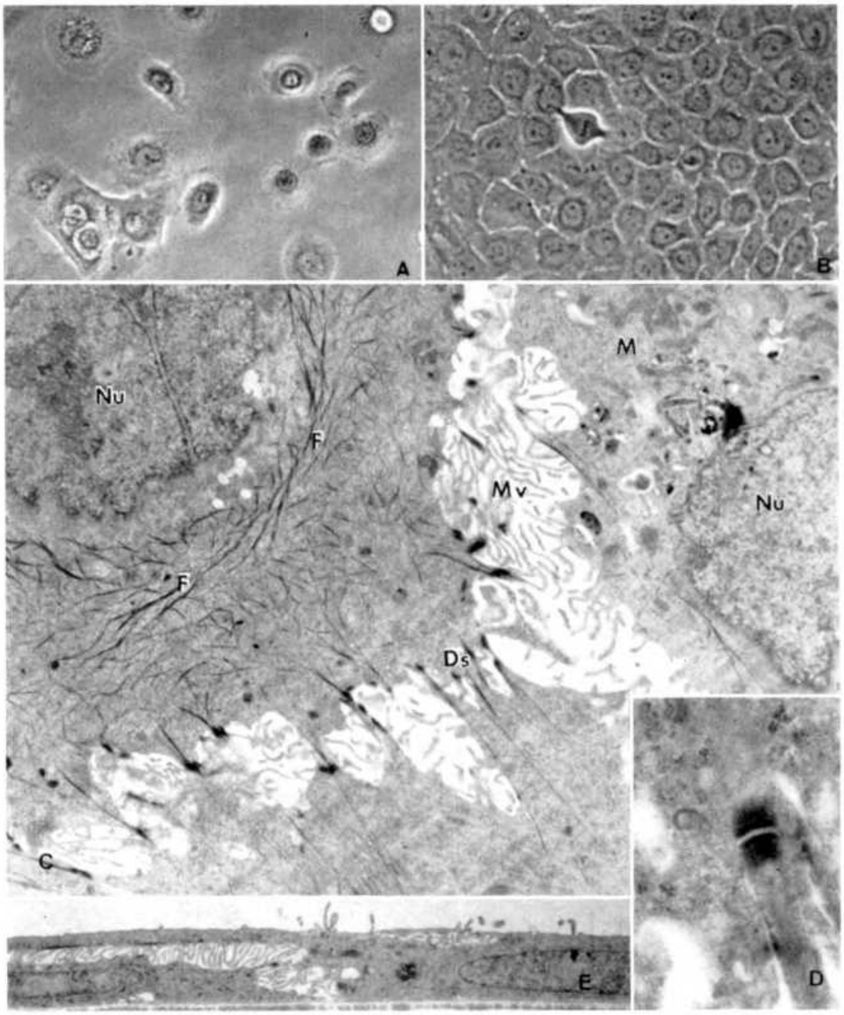

FIGURE 2. Cultured cells (HSC-1). (A) Cells of the 27th passage at 1 day after inoculation (phase contrast, × 320). (B) Cells of the 27th passage at 10 days after inoculation. They are polygonal in shape, different in size and arranged in pavement-like pattern. A mitosis is seen in the centre of the photograph (phase contrast, × 320). (C) (D) (E) The ultrastructure of the cultured cells (HSC-1) of the 25th passage: (C) and (D) are in a plane parallel to the monolayer, while (E) is in a plane perpendicular; (C) shows desmosomes (Ds), tonofilaments (F), mitochondria (M), elongated microvilli (Mv), and indented nuclei (Nu) (× 3,200); (D) a desmosome-like structure in a high power field (× 24,000); (E) two cells are overlapping each other (× 3,200).

HSC-1

Tumour cells grew steadily up to the 10th subculture in the presence of 3T3 feeders, as well as in the absence of 3T3 feeders from the 11th subculture. Figures 2A and 2B show the tumour cells of the 27th passage grown in a glass bottle observed by phase contrast microscopy. Cells were epithelioid in shape and varying in size. Electron micrographs of the cultured cells are shown in Fig. 2C, D, E. The cells had indented nuclei, filaments, elongated microvilli and desmosome-like structures. As shown in Fig. 2E, cells were grown in a monolayer. They did not pile up but overlapped each other. No evidence of keratinization, e.g. horny cells or keratohyalin granules, was obtained by electron micrography of the cultured cells.

Neither viral particles nor inclusion bodies were observed, electronmicrographically, in the tumour mass or in culture cells.

The doubling time of the culture cells (25th passage) was 43 h.

The plating efficiency of the culture cells of the 28th passage was about 65%.

The distribution of the chromosome number ranged from 40 to 162 in the cells of the 8th passage.

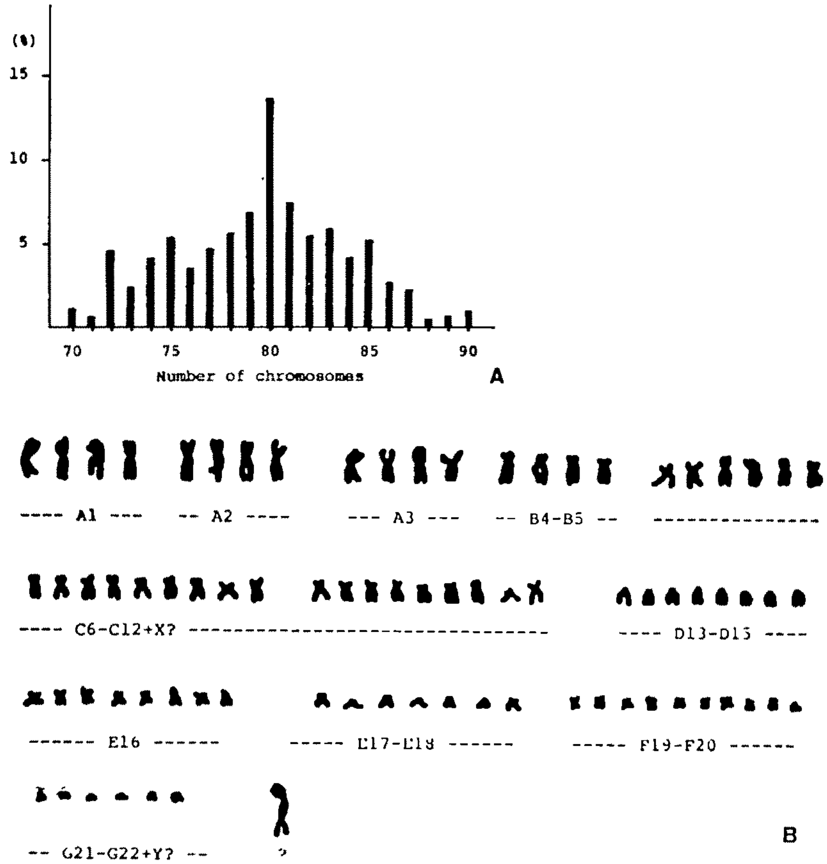

FIGURE 3. The analysis of the chromosome pattern. (A) The distribution of the chromosome number. HSC-1 cells of the 8th passage were used for the chromosome analysis. The modal number 80 was obtained (14%). (B) Metaphase with eighty chromosomes. Addition of chromosomes to each group is observed.

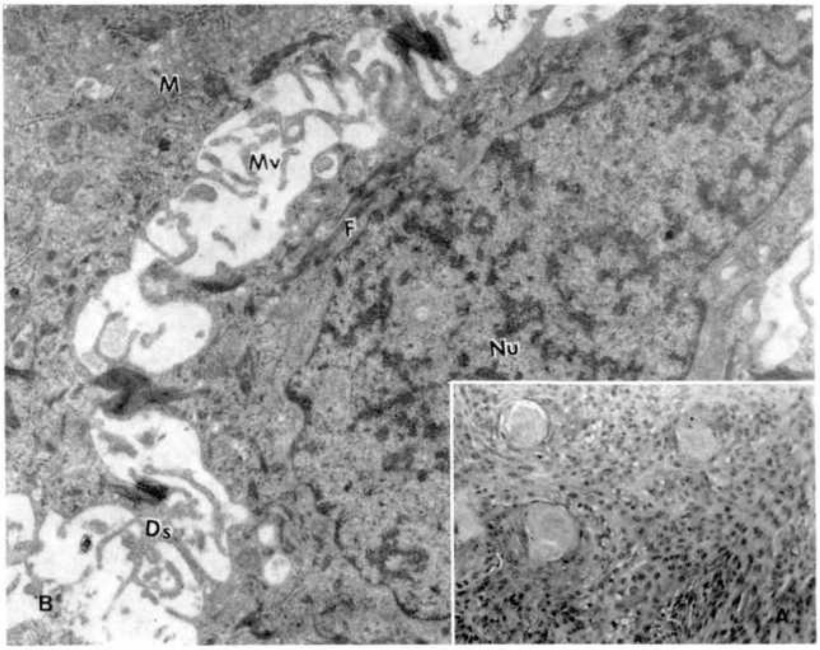

FIGURE 4. The tumour grown in a nude mouse. (A) Cells are polygonal in shape, varying in size, and horn pearls are observed frequently (H & E, × 160). (B) The ultrastructure of the tumour, showing the wide intercellular space with elongated microvilli (Mv), desmosomes (Ds), filaments (F), mitochondria (M), and a nucleus (Nu).

Figure 3A shows the distribution number between 70 and 90, with a peak at 80. No distinct marker chromosomes were observed, although some abnormal chromosomes, e.g. dicentrics and microchromosomes, were rarely found. Fig. 3B shows one of the chromosome patterns.

The tumour grown in a nude mouse was greyish in colour, invading the retroperitoneum. No necrosis was seen in the cross sections of the tumour. Figure 4A shows haematoxylin-eosin staining of the excised tumour. Cells were eosinophilic, polygonal and connected to each other by intercellular bridges. Anisonucleosis and individual cell keratinization were observed. Figure 4B shows the electron micrography of the tumour, many desmosomes and filaments being characteristically observed. The wide intercellular space with elongated microvilli is characteristic.

Cells survived the storage at −80°C. About 85% of the cells thawed after storage for 1½ years, excluded the trypan blue staining. The plating efficiency of the cells thawed was about 50%. They grew steadily forming colonies.

HSC-1b

In the primary culture, cells grew very slowly but steadily with the aid of 3T3 feeders. After the first subculture cells started to grow faster. The morphology of the culture was just like the HSC-1 cells.

Comparison between HSC-1 and HSC-1b

Table 1 shows the chromosome analyses of HSC-1 (46th passage), HSC-1b (10th passage), frozen and thawed cells (3 passages after thaw), and the tumour cells grown in a nude mouse (3rd passage).

TABLE 1. Distribution of chromosome number. A hundred cells in metaphase were chosen for counting the number of chromosomes

Cell line	Number									
	74	75	76	77	78	79	80	81	82	83
HSC-1 (46th)	4	5	7	7	6	7	12	3	4	1
HSC-1b (10th)	3	8	7	7	4	5	13	7	3	2
Frozen and thawed	6	3	6	9	8	10	14	7	5	2
Tumour in nude mouse	10	4	7	8	7	4	19	11	6	4

TABLE 2. Comparison of the doubling time. The growth rates were measured in a series of subcultures (unit = hour)

	46th	47th	50th
HSC-1	39·6	40·8	36·5
	10th	11th	14th
Frozen and thawed	41·3	43 2	39·6
	3rd	4th	7th
HSC-1b	81·6	57·6	37 2

All of them showed hypotetraploidy with the modal number 80. Table 2 shows the doubling time compared between HSC-1, HSC-1b, and frozen and thawed cells in serial cultures. In HSC-1 and frozen and thawed cells, the doubling time was around 40 h, while in HSC-1b the time became shorter, down to around 40 h according to the increase in the number of passages from 3 to 7.

DISCUSSION

Human tumour cells lines are difficult to establish. The established tumour cells might be obtained just by good fortune, and more sophisticated methods for tumour cell culture are needed in future. It has been proved that 3T3 feeders are very effective for the culture of normal human epidermal cells (Rheinwald & Green, 1975, 1977; Kondo, Aso & Namba, 1979). We used the 3T3 feeder for the culture of squamous cell carcinoma, and it has proved to be effective especially in the initial cultures, when the cells were not adapted enough to the *in vitro* conditions.

Transplantation experiments on nude mice were not successful, except for one case. It is well known that tumour cell lines are not always transplantable into nude mice. For instance, in transplantation experiments with thirty-two human cancer cell lines into nude mice, twenty-three (70%) were transplantable, and repeated transplantation experiments with non-transplantable lines were rarely successful (Oboshi & Shimosato, 1975).

During the establishing of the cell line, the doubling time of HSC-1b cells became shorter and got nearer to that of HSC-1 with increases in the number of passages.

The tumour grown in a nude mouse was again cultured and analysed for chromosome pattern, showing the same modal number as that of HSC-1, which indicates that the tumour is derived from HSC-1 cells.

HSC-1b has almost the same characteristics as HSC-1, e.g. the same modal number of chromosomes and nearly the same doubling time after the 7th passage of HSC-1b. These facts indicate that HSC-1b is derived from the same original tumour as HSC-1.

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Genome and chromosome mutations in porphyria cutanea tarda

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SUMMARY

Spontaneous genome mutations in ten patients with porphyria cutanea tarda (PCT) amounted to 1·02% as compared to 0·36% in the control group of healthy individuals ($P<0{\cdot}01$). Spontaneous structural chromosome aberrations in patients were seen in 2·39% of the examined cells versus 0·70% in the cells of controls ($P<0{\cdot}01$). The increased percentage of cells with spontaneous genome and chromosome mutations in patients with PCT cannot be associated with alcohol only.

The activity of the enzyme uroporphyrinogen decarboxylase (Uro-D) in the liver is diminished in PCT (Kushner & Barbuto, 1974). In hereditary PCT the enzymatic defect is in the liver and in the red blood cells. The enzymatic activity is decreased only in the liver in acquired PCT—a disease which may be provoked by exogenous factors such as alcohol (in 90% of the patients), salts of heavy metals, and oestrogens. Hepatomas are 130-fold more common in patients with porphyria (Berman & Braun, 1962). Hepatocellular cancer was found in about half of thirty-six patients simultaneously suffering from PCT and hepatic cirrhosis (Kordač, 1972). The tendency towards neoplastic growth in the liver draws attention to this type of porphyria from the cytogenetic point of view. Rásko, Went & Johnson (1976) established an increased repairing capacity in lymphocyte cultures from PCT patients.

We determined to study the karyotype of patients with PCT for the presence of systemic anomalies as well as to estimate the incidence and the nature of spontaneous genome and chromosome mutations in the cells.

MATERIALS AND METHODS

Examination of the karyotype was carried out in twenty-five males with PCT aged between 27 and 69 years (mean 50 years). In ten of the patients the nature and the incidence of spontaneous genome

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TABLE 1. Clinical and biological characteristics of the ten patients studied (methods: Ivanov & Drenska, 1976)

Number	Clinical stage	Aetiological factors	Urine nmol/24 h		Total serum porphyrins μg/l
			Uroporphyrin	Coproporphyrin	
8	Remission after phlebotomy treatment (Ippen, 1961)	Alcohol in 7 Alcohol + lead in 1	171 ± 129	156 + 72 (means ± s.d.)	11 ± 6
1	Active stage with fresh skin lesions	Alcohol	235	188	33
1	Biochemical relapse 3 years after phlebotomy treatment	Alcohol + lead	857	253	37

and chromosome mutations in the cells was studied (Table 1). They had not been subjected to X-ray or isotope examination for more than a year.

In five patients studied erythrocyte Uro-D was normal (Brodie *et al.*, 1977). The thin layer chromatography of porphyrin fractions in the urine after Doss (1970) revealed 8-COOH>7-COOH>4-COOH in most of the patients.

Twenty-three healthy adults from both sexes served as controls for genome mutations, and in ten of them, selected according to sex (males) and age, studies for chromosome aberrations were carried out as well. A cytogenetic analysis of the patients and the controls was carried out on short-term lymphocyte cultures, set up after the micromethod of Hungerford *et al.* (1959) with fixation being undertaken on the 72nd hour. The chromosome analysis was conducted under a light microscope with Giemsa staining. In each individual subjected to these studies the genome mutations were analysed on 100 to 300 metaphases depending upon the mitotic index, whereas the chromosome aberrations were studied on 50 to 120 mitoses. We did not include gaps in the number of chromosome aberrations. The statistical evaluation was performed after the criterion of χ^2 and the exact probability test of Fisher.

RESULTS

No systemic deviations in the karyotype were detected in the karyological analysis of the patients with PCT. They all had a karyotype of 46 XY. Spontaneous genome mutations are more common in PCT patients as compared with the controls ($\chi^2 = 6{\cdot}23$; $P<0{\cdot}02$; exact probability test of Fisher—$P = 0{\cdot}011$) (Table 2). The difference between the patients with PCT and the control group is also

TABLE 2. Spontaneous genome mutations

	Number of examined persons	Persons with genome mutations		Examined cells	Cells with genome mutations	
		Number	%		Number	%
Patients with PCT	10	8	80	2540	26	1·02
Controls	23	6	26	3100	11	0·36

significant ($\chi^2 = 8{\cdot}58$; $P<0{\cdot}01$) in respect to the number of the mutant cells. The genome mutations represented polyploidy of the type of chromosomes (with a number of 4n) chaotically dispersed in the metaphase. Endoreduplication was found in only one of the examined metaphases (in cells from PCT patients), which was equal to 0·4% of all the examined cells.

The spontaneous structural chromosome aberrations detected in the group of patients significantly outnumber those detected in the controls ($\chi^2 = 7{\cdot}78$; $P<0{\cdot}01$) (Table 3). The established chromosome aberrations are of the type of chromatids and chromosome breaks with a prevalence of the first. Two centromeric breaks were also seen in the group of patients.

TABLE 3. Spontaneous structural chromosome aberrations

	Number of examined persons	Examined cells	Cells with aberrations		Number of aberrations	Aberration in a single cell
			Number	%		
Patients with PCT	10	837	20	2·39	20	0·0239
Controls	10	995	7	0·70	8	0·0070

DISCUSSION

The results of our studies reveal that spontaneous genome as well as chromosome mutations in peripheral blood lymphocytes from patients with PCT exceed those in healthy controls. As factors inducing these changes, the intake of alcoholic beverages, the altered metabolism of porphyrins or their combined effect could be considered. Several authors have reported a mutagenic effect of alcohol in chronic alcoholics as well as in the rat (Ilinskikh *et al.*, 1978; Mitelman & Wadstein, 1978; Obe & Herha, 1975), but some differ (Cadotte, Allard & Verdy, 1973; Shapiro, Sicheva & Grinberg, 1974). As far as our ten patients with PCT are concerned, they all belong to the group of social drinkers and no one of them could be considered as a chronic alcoholic. Bearing in mind the high incidence of hepatomas in patients with PCT and liver cirrhosis, which was not so high in chronic alcoholics with liver cirrhosis (Lowenfels, 1979), it seems much more probable that the observed spontaneous genome and chromosome mutations in the lymphocyte cultures do not occur solely as a result of the effect of alcohol. Further studies are warranted on their association with some metabolites of the disturbed porphyrin metabolism as a mutagenesis-inducing factor.

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Analysis of lipids and dioxin in chloracne due to tetrachloro-2,3,7,8-p-dibenzodioxin

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SUMMARY

Biochemical investigations on the lipid and dioxin content of the cysts and comedo-like lesions of three children exposed to tetrachloro-2,3,7,8-p-dibenzodioxin (TCDD) have been performed.

Gas chromatography and mass spectrometry failed to demonstrate the presence of dioxin despite the high sensitivity of the method. Further investigation by thin layer and gas chromatography-mass spectrometry showed that the lipids of the chloracne lesions in these children were exclusively epidermal in origin.

These findings support the hypothesis that exposure to dioxin produces a hyperproliferative reaction of the cutaneous epithelium with squamous metaplasia of the cells lining the ducts of skin glands.

Tetrachloro-2,3,7,8-p-dibenzodioxin (TCDD) is formed coincidentally during the alkaline hydrolysis of tetrachlorobenzene to produce chlorphenate, which is used as an insecticide and herbicide. Chloracne is the cutaneous response to certain chlorinated aromatic substances, of which TCDD is the prototype compound. Despite our detailed knowledge of the clinical features, and the chemical structure of the responsible compounds (Crow, 1970; Jensen, Sneddon & Walker, 1971), the pathogenesis of the disease remains obscure.

After an episode of accidental TCDD intoxication occurred at Seveso, Italy, in 1976, one of us (Gianotti, 1977) observed several children with chloracne. In this paper, the absence of dioxin from chloracne lesions and the biochemical analysis of their lipid content are reported and discussed.

METHODS

We studied three children with chloracne following contamination with TCDD: a 4-year-old boy, and two sisters aged 4 and 2 years respectively. The children were first observed a few days after the exposure to TCDD. The first histological examination was performed in the boy after 20 days, and in the two sisters after 3 months. The clinical and histological features have been reported elsewhere (Gianotti, 1977).

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Five months after the contamination, lesions were removed from all three children and their lipid content investigated. In the two sisters a further test was performed 11 months after the exposure.

Cysts and comedo-like lesions were isolated from the material obtained by curettage from face, ears and axillae. The samples were weighed (5·10 to 7·30 g in each case) and stored at −25°C until analyzed.

Dioxin determination

Two-thirds of the total material was used for dioxin determination. The material was homogenized, dehydrated in a desiccator on phosphorus pentoxide and transesterified by refluxing for 3 h with 2% (w/v) concentrated sulphuric acid in redistilled methanol (Boniforti *et al.*, 1973). Fatty acid methylesters and dioxin possibly present were extracted with anhydrous redistilled benzene; they were concentrated and then injected directly in the gas chromatographic inlet system of the mass spectrometer.

Mass spectra were obtained on the LKB Mod 9000 S combination GC-MS. The gas chromatographic inlet system was equipped with a 2 m coiled glass column, inner diameter 3 mm, packed with OV 61 3% on Chromosorb W HP 80–100 mesh. Carrier gas: helium at flow rate of 20 ml/min. Column temperature: 250°C; injector: 280°C. The molecular separator was maintained at 250°C and the ion source at 270°C.

Standard of tetrachloro-2,3,7,8-p-dibenzodioxin showed a retention time of 12 min. Mass fragmentography: TCDD focused masses: m/e 320, 322, 324. The sensitivity allowed the detection of dioxin in quantities lower than 50 pg with a signal to noise ratio higher than 4.

Lipid investigations

Lipid investigations were performed by using the technique previously applied to study the effect of ageing on fatty acids of skin surface lipids (Nazzaro-Porro *et al.*, 1979).

Extract of lipids. One third of the total material was used for lipid investigations. The bulk of the content from cysts and comedo-like lesions was obtained by extrusion and the remainder by scraping of the sac walls. During the removal efforts were made to avoid contact with subcutaneous fat. Extraction of lipids was performed with 40 ml of chloroform-methanol (2:1, v/v) for 3 h at 40°C and twice with 10 ml of chloroform-methanol (2:1, v/v) for 1 h at room temperature. Pooled extracts were dried over anhydrous sodium sulphate and lipids recovered by evaporation of solvents under reduced pressure on a rotary evaporator below 40°C.

The total lipid extracts were weighed (they represented 10–12% of the starting material weight) and then separated into their different fractions by thin layer chromatography (TLC) (Passi *et al.* 1977). The lipids on the developed chromatograms were detected by two methods: (a) charring by heating up to 150°C with 50% v/v sulphuric acid for densitometry (Nazzaro-Porro *et al.*, 1973); (b) staining with bromcresol green solution for gas liquid chromatography (GLC) (Boniforti *et al.*, 1973). The lipid spots corresponding to free fatty acids (FFA), triglycerides (TG) and sterol esters (SE) were scraped off separately from the plate and the scrapings were extracted three times with peroxide-free diethyl-ether. The recovered lipid solution was dehydrated with anhydrous sodium sulphate and evaporated nearly to dryness under a nitrogen stream.

Gas liquid chromatography

The FFA fraction was methyl esterified with diazomethane; TG and SE were separately transesterified by refluxing for 3 h with 2% (v/v) concentrated sulphuric acid in redistilled methanol. Fatty acid methylesters of SE fraction were purified from sterols by TLC (Nazzaro-Porro *et al.*, 1979). An aliquot of FA methylester mixture of each fraction was used for gas liquid chromatography on packed column, and a capillary column was used to detect the presence of branched fatty acids. Within the branched fatty acids we were capable of identifying only the isobranched saturated (by comparison with standard reference compounds, Analabs Inc.) and isobranched unsaturated (by hydrogenation). Other positions of the methyl group in branched chains were not studied due to lack of reference standards.

Packed column. A stainless column (4 m × 2·5 mm internal diameter) containing Apiezon L as stationary phase was applied to a Dani 3900 gas chromatograph equipped with a hydrogen flame ionization detector. Helium at a flow rate of 1 ml/min was the carrier gas, splitter ratio: 1:50. The operating temperatures were: column isothermal 175°C; injector 270°C; detector 200°C.

Double bond position of unsaturated fatty acids.

(1) Performic acid peroxidation of fatty acid methylesters: the remaining fatty acid methylester mixtures of each of the three fractions (triglycerides, free fatty acids and sterol esters) were weighed before conversion to diols or polyhydroxymethylesters. Each mg of the samples was allowed to react with 15 μl of formic acid and 2 μl of 30% hydrogen peroxide at 40°C for 2 h with occasional shaking. The reaction mixture was diluted with 1 ml distilled water, extracted three times with 5 ml portions of peroxide-free redistilled diethyl-ether, the extract pooled and the ether evaporated to dryness under nitrogen stream. The product was saponified in 0·1 ml 3N sodium hydroxide at 100°C for about 30 min, acidified with diluted sulphuric acid and extracted three times with 5 ml portions of peroxide-free diethyl-ether. The pooled extracts, containing saturated dihydroxy and polyhydroxy fatty acids, were evaporated to dryness under a nitrogen stream.

(2) Formation of trimethylsilyloxy derivatives: The previous mixtures were methylated with diazomethane. The corresponding dihydroxy fatty acid methylesters were converted to the respective trimethylsilyloxy derivatives (TMSO) by treatment with 0·2 ml of N,O-bis-(trimethylsily)-acetamide (BSA) and trimethylchlorsilane (TMCS) (5:1) in pyridine. The mixtures were allowed to stand for 5 min and injected directly in the gas chromatographic inlet system of the mass spectrometer. Mass spectra were obtained on LKB Mod 9000 S combination GC-MS. The gas chromatographic inlet system was equipped with a 3 m coiled glass column packed with 5% OV-101 in Chromosorb W HP 80–100 mesh. Carrier gas: helium at flow rate of 25 ml/min. Column temperature was initially isothermal at 180°C for 10 min and then the column temperature was programmed from 180°C to 230°C at the rate of 3°C/min and isothermally thereafter. The He separator was maintained at 250°C and the ion source at 270°C. Ionizing potential was 20 eV and the ionizing current was 60 mA. Average spectra from respective scanning were recorded for each gas chromatographic peak.

Gas liquid chromatography of sterols. The dry sterol extracts were converted to the corresponding TMSO derivatives (trimethylsilyloxy) by treatment with 0·5 ml of a pyridine-hexamethyldisilazone (HMDS)-trimethylchlorsilane (TMCS) (103:3:1). The mixture was allowed to stand for 5 min and injected directly in a Carlo Erba Mod.GV equipped with a hydrogen flame ionization detector. A 3 m coiled glass column packed with 3% OV 17 on silanized acid-washed Chromosorb W HP 80–100 mesh, was applied to the instrument. Flow rate was 30 ml/min of nitrogen carrier gas. The operation temperatures were: column isothermal 250°C; injector 280°C; detector 230°C.

RESULTS

Dioxin determination

No dioxin was detected by the GC-MS analysis of lipid material.

Lipid investigation

Table I shows the different classes of lipids obtained by TLC. It is evident that wax esters and squalene were absent, while sterols and sterol esters were very relevant, representing 65% of total lipids. The gas chromatographic analysis of sterols and sterol esters showed that the main sterol component, in both the fractions, was cholesterol (99%), lathosterol being present only in traces. The free fatty acid fraction was well represented, while triglycerides were in low proportion. There was no significant difference in the lipid composition of cysts and comedo-like lesions analysed at different times after exposure to TCDD.

TABLE 1. Percentage lipid composition of chloracne lesions in children exposed to TCDD

Cases	Time from exposure	DG (1–2, 2–3, 1–3)	ST	FFA	TG	WE	SE	SQ
1.	5 months	1·8	44·2	22·2	7·9	—	23·8	tr
2.	5 months	3·3	36·8	25·7	9·8	—	24·3	tr
	11 months	3·6	44·7	21·3	8·9	—	21·4	tr
3.	5 months	1·8	43·4	17 7	9 3	—	27·7	tr
	11 months	3·0	46·9	18·8	11·6	—	19·6	tr

DG, diglycerides; ST, sterols; FFA, free fatty acids; TG, triglycerides; WE, wax esters; SE, sterol esters; SQ, squalene.

Phospholipids were not considered; because of their polarity they remain at the origin.

Analytical data on fatty acid composition and double bond position of the unsaturated fatty acids of free fatty acids, triglycerides and sterol esters classes of lipids of chloracne of the three children are not reported here in detail; the relative amounts of the different fatty acid types of free fatty acids, triglycerides and sterol esters are reported in Table 2. In all the three classes of lipids we can observe

TABLE 2. Relative amounts of the different fatty acid types of FFA, TG and SE classes in chloracne lipids and their $\Delta 6$ and $\Delta 9$ patterns

Lipid classes	Straight fatty acids mean%					Branched fatty acids mean %			Others§ mean%
	Saturated	Monounsaturated		Polyunsaturated			Unsaturated		
		$\Delta 6$	$\Delta 9$	$\Delta 6$	$\Delta 9$	Saturated	$\Delta 6$	$\Delta 9$	
Free fatty acids	32·99	tr	26·58	tr	26·65*	tr	—	—	13·68
Triglycerides	35·12	tr	27·34	—	18 73†	tr	—	—	18·71
Sterol esters	24 66	tr	27·66	—	24·44‡	tr	—	—	23 14

Relative amounts of the different fatty acid types were calculated by the results obtained by capillary and packed column. The structure of the unsaturated fatty acids was studied by GLC-MS analysis of TMSO derivatives of fatty acid methylesters (for details see text).

tr = <0.1%.

* C18:2, 13·76%; C18:3, 2 48%; C20:2, 1·77%; C20:4, 4·55%.

† C18:2, 12·30%; C18:3, 3·45%; C20:2, 0·65%; C20:4, 2·35.%.

‡ C18:2, 14·77%; C18:3, 4·12%; C20:2, tr; C20:4, 9·15%.

§ Others were constituted by fatty acids with chain length over C20:4 which have not been identified due to lack of standards.

that: (a) the branched fatty acids, either saturated or unsaturated, were present only in traces ($<0{\cdot}1\%$) or absent, (b) within the straight fatty acids a very high percentage was represented by polyunsaturated fatty acids—the 'others' were constituted by fatty acids with chain length over C20:4 which have been calculated but not identified due to lack of standards; (c) all the unsaturated fatty acids, from the lowest to the longest chains, had a Δ 9 pattern, and C18: 2 was exclusively represented by C18:9–12, i.e. linoleic acid.

DISCUSSION

The GC-MS analysis of lipids extracted from the pooled material obtained by curettage of chloracne lesions in children exposed to TCDD failed to demonstrate the presence of dioxin. The fact that dioxin was not detected despite the high sensitivity of the method (the weight of starting material from each case varied from 3·40 to 4·86 g and the method allowed the detection of dioxin in quantities lower than 50 pg) might be related to the time elapsed between the contamination and the investigation. If the substance is still present, it does not appear to be excreted in skin lipids.

The lipid investigations were performed in order to determine whether the lipids were of sebaceous and/or epidermal origin. As reported by Crow (1970), the clinical and experimental data in adult humans and in animals suggest that comedones and cysts of chloracne are due to a keratinizing metaplasia of the epithelial lining of the pilosebaceous follicles associated with precocious atrophy of sebaceous glands (Shelley & Kligman, 1957); in the experimental rabbit it has been shown that sebaceous gland atrophy even precedes the development of an occluded comedo (Hambrick, 1957). Other authors (Cunliffe *et al.*, 1975) suggest that chlorinated hydrocarbons induce chloracne by modifying the metabolism of the pilosebaceous follicle.

The lipids of epidermal origin differ greatly from those derived from sebaceous glands. Most studies in this field have been carried out by analysing skin surface lipids which are derived mainly from the sebum with a small proportion from the Malpighian layer of the epidermis. The relative contribution of lipids from each of these sources depends upon the degree of activity of sebaceous glands which, being under hormonal, and especially androgenic control (Ebling, 1974; Pochi & Strauss, 1974), varies with age and sex. Prepubertal children and old people have a low sebaceous activity as compared with young or adult subjects; nevertheless sebaceous lipids are always present in their skin surface lipids indicating a continuous activity of sebaceous glands (Cotteril *et al.*, 1972; Nazzaro-Porro *et al.*, 1979).

In addition to the studies on skin surface lipids at different ages and on cutaneous areas more or less rich in sebaceous glands (Greene *et al.*, 1970), investigations on whole isolated sebaceous glands (Kellum, 1967) and on isolated epidermis (Ansari, Nicolaides & Fu, 1970) have been performed. These studies have shown that the lipid classes characteristic of sebum are wax esters and squalene; that sterols are in high prevalence in lipid of epidermal origin and that they are represented mainly by cholesterol, that sterol esters are formed by esterification of cholesterol with fatty acids coming both from epidermis and sebaceous glands (Freinkel & Aso, 1969; Nicolaides, 1963) and that triglycerides and free fatty acids are of both sebaceous and epidermal origin.

The researches on fatty acid composition of the different lipid classes (Nicolaides & Wells, 1957; Downing & Strauss, 1974; Nikkari, 1974; Nazzaro-Porro *et al.*, 1979) have shown that epidermis and sebaceous glands can make the same kind of fatty acids but in widely different amount. Sebum has a significant amount of fatty acids not commonly found in nature as odd chain length fatty acids or branched fatty acids; in addition, the level of unsaturation of the unsaturated fatty acids of sebum characteristically presents a $\Delta 6$ pattern, which is very rare in nature. Epidermis produces less odd and branched fatty acids than sebum and, as in most living tissues, most of the unsaturated fatty acids have a Δ9 pattern. Within the polyunsaturated fatty acids present on the skin surface, the most abundant is represented by C18:2, the others being present only in a very low proportion. C18:2 presents two isomers: C18:9–12, the essential fatty acid 'linoleic acid' and C18:5–i, characteristic of sebaceous lipids, named in fact 'sebaleic acid' (Nicolaides, 1974).

In our investigations, the TLC analysis of lipid in cysts and comedo-like lesions of children with chloracne showed the complete absence of the characteristic sebaceous lipid (wax esters and squalene), and a high content of epidermal lipid (sterols and sterol esters), the main sterol being cholesterol. These findings are similar to those reported in a study of lipid content of keratinous cysts of epidermis (Nicolaides, 1968) but different from those obtained from comedones of acne vulgaris in which sebaceous lipids are always present (Nicolaides *et al.*, 1970; Nazzaro-Porro *et al.*, 1973).

From Table 1 it is also evident that free fatty acids occur in high percentage while the triglycerides are at a low level. In skin surface lipids and in comedones from acne vulgaris, the free fatty acids are mainly generated by the hydrolysis of triglycerides due to the lipolytic activity of cutaneous skin flora (Kellum, 1967; Nicolaides & Wells, 1957; Nicolaides, 1973). Recent experiments, however, indicate that during epidermal keratinization a process of rescue of lipids liberated by the degenerating corneum cell membranes takes place, the neutral lipids becoming esterified with fatty acids derived from hydrolysis of phospholipids (Freinkel, 1979). That a similar mechanism occurs in the genesis of free fatty acids in chloracne is suggested by the strong similarity in the fatty acid composition of the three fractions, triglycerides, free fatty acids and sterol esters (Table 2). The epidermal origin of all three lipid fractions is revealed by the gas chromatographic analysis showing the absence, or the presence only in traces, of branched fatty acids and the very high percentage of polyunsaturated fatty acids, many of which have a chain length over C18:2. It is further confirmed by the study of the double bond position of the unsaturated fatty acids showing that all the unsaturated fatty acids, from the shortest to the longest chains, have a Δ9 pattern, and that the more representative C18:2 is exclusively formed by C18:9–12, linoleic acid (Table 2).

The analysis of the composition and structure of lipids of chloracne in our children gave similar results in all the investigations performed in spite of the different features of the cutaneous lesions. These varied, according to the time elapsed from the contamination with TCDD, from severe and diffuse hyperkeratotic lesions with granulomatosis, to cystic masses and follicular comedo-like hyperkeratosis (Gianotti, 1977). The biochemical data, demonstrating the epidermal origin of lipid content of cutaneous lesions, were in line with the histological findings, which showed the presence of epidermal hyperplasia with hyperkeratosis and a squamous metaplasia of the cells lining the duct of the sweat glands (Gianotti, 1977). According to the data reported in adult chloracne, in which the squamous metaplasia affects mainly the cells of the sebaceous gland duct, the cutaneous pathology induced by exposure to dioxin seems to be related to a hyperproliferative reaction of the entire cutaneous epithelium. Whether the squamous metaplasia is a temporary condition, due to the direct toxic effect of dioxin, will be clarified only after long term observation.

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Decreased lymphocyte reactivity and auto-immunity in alopecia areata

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SUMMARY

T lymphocyte numbers and functions were measured in forty-six patients with alopecia areata and thirty healthy controls. In patients with alopecia areata, lymphocyte reactivity to extracts of scalp and hair follicles was not detected by ^{3}H thymidine incorporation. ^{3}H Thymidine incorporation by lymphocytes cultured with PPD, Varidase and *C. albicans* was significantly reduced in cells from patients compared with controls and correlated with the extent of hair loss and the presence of anti-thyroid antibodies. Decreased lymphocyte responses to PPD and Varidase were not due to the presence of suppressor monocytes because removal of monocytes with carbonyl iron did not increase lymphocyte reactivity. However, responsiveness to *C. albicans* was augmented by removal of monocytes.

T cell numbers were significantly lower than in controls ($P<0\cdot005$); patients with auto-antibodies had fewer T cells than patients without them.

The relationship of reduced T cell function and auto-immunity to alopecia areata is discussed.

The prevalence of auto-antibodies against thyroid constituents, gastric parietal cells and smooth muscle is increased in alopecia areata (Kern *et al.*, 1973; du Vivier & Munro, 1975; Main *et al.*, 1975; Friedmann, 1981) and there is an increased prevalence of thyroid disease and diabetes in patients and their relatives (Muller & Winkelmann, 1963; Cunliffe *et al.*, 1969; Main *et al.*, 1975). Attempts to demonstrate antibodies to hair follicles have proved unsuccessful (Kern *et al.*, 1973; Klaber & Munro, 1978; Bystryn, Orentreich & Stengel, 1979; Igarashi, Takeuchi & Sato, 1980) and the C3 complement found round hair follicles (Bystryn *et al.*, 1979; Igarashi *et al.*, 1980) is probably non-specific. It therefore seemed worthwhile examining cell-mediated reactivity to follicle antigens. In addition, since reduced T cell numbers have been reported (Brown, Olkowski & McLaren, 1977; Gianetti *et al* 1978) T cell function was assessed by measurement of responses to microbial antigens.

MATERIALS AND METHODS

Patients

Forty-six patients with alopecia areata (mean age 34·3 years, s.d. 14) and thirty healthy control subjects (mean age 32·3 years, s.d. 14) were studied. The extent of the disease ranged from small active

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patches in twenty to complete or almost complete hair loss in twenty-six patients. The duration of disease ranged from 2 months to 20 years. Auto-antibodies against thyroid, gastric parietal cells, smooth muscle and a variety of other antigens (Friedmann, 1980) were looked for in patients and controls.

Antigens

Follicle antigens.

(1) Cadaver scalp was shaved, the epidermis was removed with an electrokeratotome and dermal fat was pared away to just below the lower ends of the follicles. The remaining follicle-bearing zone of dermis was homogenized in a freezer-mill and extracted overnight in cold phosphate buffered saline (PBS) pH 7·2. Extracts were also made in the presence of sodium deoxycholate (10 mg/ml) which was subsequently removed by dialysis against PBS. Coarse debris was removed by low speed centrifugation. Fine debris was sedimented and the supernatant was used after resuspension in PBS.

(2) Similar extractions were performed on follicles obtained by plucking hairs from healthy volunteers.

(3) Autologous scalp: scalp biopsies were taken under local anaesthesia from the margins of lesions of alopecia areata in three patients. Part of each biopsy was processed for histological examination; the remainder was finely sliced and used as antigen in tests on patients' own lymphocytes. Some cultures contained scalp alone, others contained scalp and lymphocytes.

Other antigens.

(1) Purified protein derivative of tuberculin (PPD) (Weybridge) was dissolved in PBS. After preliminary experiments the optimal concentration was found to be 10 μg/ml.

(2) Streptokinase/streptodornase ('Varidase', Lederle) was dissolved in PBS and used at 50 units/ml.

(3) *Candida albicans* blastospores were scraped from blood agar plates, suspended in PBS at 10^9/ml, killed by heating at 60°C for 30 min and used at a final concentration of 10^8/ml.

Lymphocyte culture

Peripheral venous blood was taken into syringes which contained 10 units/ml of preservative-free heparin (Evans). Undiluted blood (7 ml) was layered onto a mixture of Ficoll/Isopaque (S. G. 1·077) and centrifuged at 400 *g* for 20 min. Bands of lymphoid cells were collected, washed thrice in cold PBS and resuspended at 10^6/ml in RPMI 1640 (Flow) supplemented with 10% pooled normal human serum (NHS). Cells were dispensed in 250 μl volumes into flat-bottomed wells of microtitre trays (Linbro). Twenty-five microlitres of appropriate antigen solution was added to triplicate wells; control wells received 25 μl of medium alone.

Cultures were incubated at 37°C for 6 days in a humidified atmosphere of 5% CO_2 in air. ^{3}H Thymidine (0·2 μCi per well, 2 Ci/Mmol, Amersham) was added for the last 18 h. Cultures were terminated by aspiration onto glass fibre discs using a cell harvester (Mash II). The discs were washed, dried and counted in a liquid scintillation counter (Packard). The geometric mean of counts per minute for replicate cultures was calculated.

Enumeration of T lymphocytes

Sheep red blood cells (SRBC) (Flow) were washed thrice in PBS and resuspended at 0·5% v/v. Fetal calf serum (FCS) was heat inactivated for 30 min at 56°C and absorbed twice with SRBC. Lymphoid cells (100 μl at 3×10^6/ml) were added to 50 μl of SRBC suspension and 50 μl of FCS. After incubation at 37°C for 10 min the mixture was centrifuged gently (50 *g* for 4 min) and placed in an icebath for 2 h. The cell mixture was gently resuspended by rotating the tubes about their long axis. A drop of brilliant cresyl blue was added to stain the lymphoid cells, 200 of which were counted in a haemocytometer. The proportion which had formed rosettes with at least 3 SRBC was counted. The mean of duplicate tubes was calculated and the absolute number of T cells in millions per ml was calculated from the total and differential white cell count performed on the blood.

Depletion of monocytes

The concentration of lymphoid cells was adjusted to $3{\cdot}5 \times 10^6$/ml in RPMI with 20% normal human serum. Sixty mg of carbonyl iron (GAF) was added to 10 ml of cell suspension. The mixture was shaken on a 'Matburn' mixer at 37°C for 30 min. The iron was pulled to the bottom of the tube with a strong magnet and the supernatant removed. The procedure was repeated once. The cells were washed once, resuspended at 10^6/ml and placed in culture alongside cells which had not been treated with iron.

Results and statistics

Geometric mean counts per minute ± s.e.mean were calculated for each group of subjects. Student's t test was used to compare means of samples.

RESULTS

Lymphocyte reactivity to follicle extracts

Serial dilutions of extracts prepared from either cadaver scalp or plucked hair roots were added to cultures of lymphocytes from patients with early active alopecia or long established disease. Higher concentrations had non-specific inhibitory effects while lower concentrations failed to stimulate. In three cases, fine slices of scalp containing approximately equal numbers of follicles were placed in

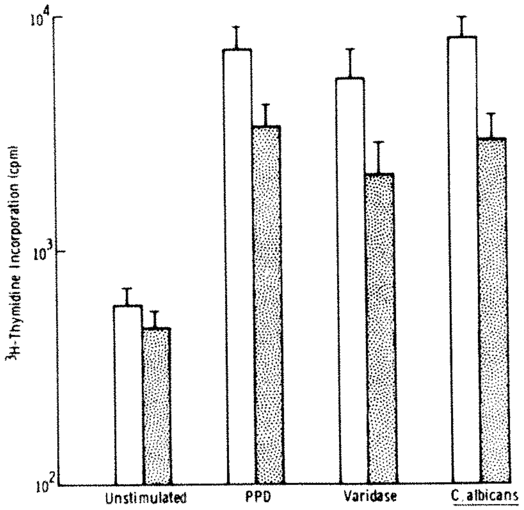

FIGURE 1. Lymphocyte responses to microbial antigens: comparison of patients with alopecia areata and healthy controls. Unshaded bar shows geometric mean counts/min ± s.e.mean for control subjects. Shaded bar shows geometric mean counts/min ± s.e.mean for alopecia patients. Unstimulated: thirty controls, forty-six patients, difference not significant (NS). PPD: thirty controls, forty-six patients, $P < 0{\cdot}02$. Varidase: thirteen controls, twenty-three patients, $P < 0{\cdot}05$. *C. albicans*: twenty-four controls, thirty-seven patients, $P < 0{\cdot}005$.

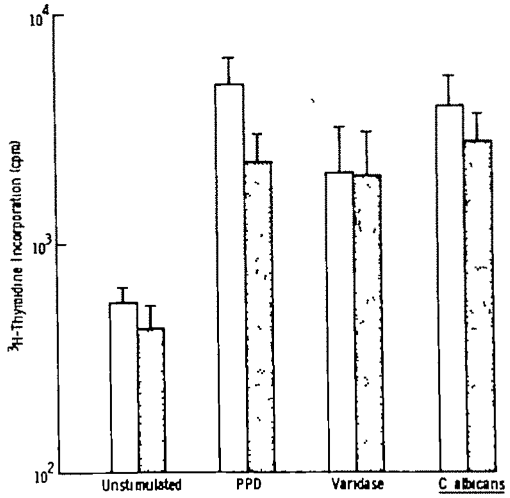

FIGURE 2. Lymphocyte responses to microbial antigens: comparison of patients with total and partial alopecia. Unshaded bar shows patients with partial alopecia (geometric mean counts/min ± s.e.mean). Shaded bar shows patients with total alopecia. Unstimulated: twenty partial alopecia, twenty-six total alopecia, NS. PPD: twenty partial alopecia, twenty-six total alopecia, $P < 0 \cdot 05$. Varidase: seven partial alopecia, fifteen total alopecia, NS. *C. albicans*: twelve partial alopecia, twenty-three total alopecia, NS.

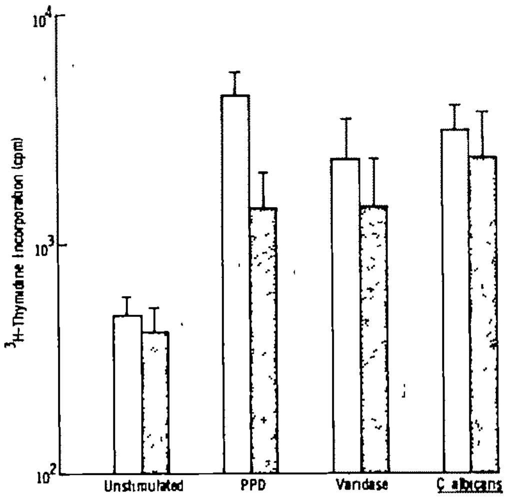

FIGURE 3. Lymphocyte responses to microbial antigens for patients with alopecia areata: comparison of patients with and without anti-thyroid antibodies. Unshaded bar shows patients without antibodies (geometric mean counts/min ± s.e.mean. Shaded bar shows patients with antibodies. Unstimulated: thirty-three without antibodies, thirteen with antibodies, difference NS. PPD: as for unstimulated, $P < 0 \cdot 01$. Varidase: thirteen without antibodies, nine with antibodies, NS. *C. albicans*: twenty-five without antibodies, eleven with antibodies, N.S.

replicate wells of microtitre trays. The patients' own lymphocytes were added to some wells. Incorporation of ^{3}H thymidine in cultures which contained scalp alone was subtracted from that given by lymphocytes and scalp: no stimulation of lymphocytes was detected.

Lymphocyte responses to microbial antigens

When cultured without added antigen, lymphocytes from patients with alopecia gave slightly lower spontaneous incorporation of ^{3}H thymidine than cells from controls but the difference was not significant (Fig. 1). When cultured with different microbial antigens, however, lymphocytes from patients with alopecia gave significantly lower responses than cells from healthy control subjects: $P<0\cdot02$ for PPD, $P<0\cdot05$ for Varidase, and $P<0\cdot005$ for *C. albicans* (Fig. 1). The reductions were greatest in patients with extensive hair loss although only the responses to PPD were significantly different ($P<0\cdot05$) (Fig. 2).

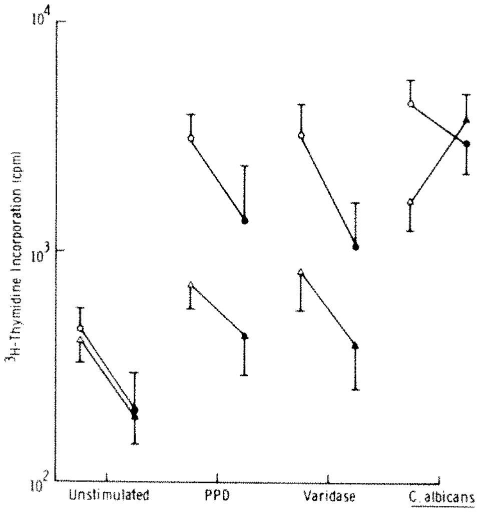

FIGURE 4. Effect of depletion of monocytes on lymphocyte responses to microbial antigens: ○ = controls, n = 12; △ = low responder patients (see text for details), n = 11. Solid symbols are responses after depletion of monocytes. Points are geometric mean counts/min, bars are s.e.mean.

To see if reduced lymphocyte responsiveness was correlated with other immunological derangements, responses of cells from patients with anti-thyroid antibodies were compared with those of cells from patients without antibodies. Both spontaneous and antigen-induced ^{3}H thymidine incorporation was lower in cells from patients with anti-thyroid antibodies (Fig. 3). The reduction was significant for PPD-induced responses ($P<0\cdot01$) but not for Varidase or *C. albicans*.

Effects of depletion of monocytes upon lymphocyte responses

Experiments were performed to examine the possibility that very low lymphocyte responses (less than 4000 counts/min, stimulation ratio less than 3·5) given by some patients were due to the presence of 'suppressor' monocytes. After lymphoid cells were separated, one aliquot was placed in culture as usual; the remainder were depleted of monocytes with carbonyl iron before being placed in parallel cultures. Cells from eleven unresponsive patients and twelve controls were tested. After depletion of monocytes, similar reductions were observed in responses to PPD and Varidase by lymphocytes from patients and controls (Fig. 4). Responses of cells from control subjects to *C. albicans* were also reduced by monocyte depletion but, in contrast, lymphocytes from seven of eleven patients with alopecia gave increased responses to that antigen.

Of the eleven patients who were particularly unresponsive, six had anti-thyroid antibodies and one had anti-smooth muscle antibodies.

T cell numbers

T cell numbers were measured in seventeen patients with alopecia (mean age 38·3 years, s.d. 16) and compared with those in sixteen control subjects (mean age 41·6 years, s.d. 13). The mean number of T cells for the patients ($1{\cdot}05 \times 10^6$/ml$\pm 0{\cdot}32$ s.d.) was significantly ($P<0{\cdot}005$) lower than that for controls ($1{\cdot}64 \times 10^6$/ml$\pm 0{\cdot}57$ s.d.). Of the seventeen patients examined, eleven had auto-antibodies. The mean T cell number in these patients was $0{\cdot}96 \times 10^6$/ml compared with $1{\cdot}25 \times 10^6$/ml for patients without such antibodies. The difference was not significant but this probably reflects the small numbers of the sample.

DISCUSSION

The results presented here confirm previous reports that T cell numbers are reduced in alopecia areata (Brown, Olkowski & McLaren, 1977; Gianetti *et al.*, 1978). Patients with the lowest T cell numbers were those who also had anti-thyroid antibodies. The results also show decreased T cell reactivity to microbial antigens. Diminished lymphocyte responses, particularly to PPD, were associated with the presence of auto-antibodies and showed some correlation with the extent of alopecia. However, this association was not strong and very low lymphocyte responses were seen in some patients with only small areas of hair loss. This suggests that the decreased T cell responsiveness is not merely a secondary effect of a severe form of the disease but rather reflects some other disturbance of the immune system.

The antigen-induced proliferation of lymphocytes is a recognized indicator of T cell function (Valentine, 1971). However, correlation between low T cell numbers and responses failed to attain significance ($0{\cdot}1 < P > 0{\cdot}05$) and it seems, therefore, that low T cell numbers cannot entirely account for the reduced responses. It seems unlikely that decreased lymphocyte responses to PPD and Varidase were due to the presence of suppressor monocytes since their depletion with carbonyl iron did not augment reactivity to those antigens. In contrast, suppressor monocytes appear to be implicated in the reduced responses to *C. albicans* since increased lymphocyte responses were obtained after iron treatment. It is not clear why suppressor monocytes should act in one system rather than the others but *C. albicans* is a particulate antigen whereas PPD and Varidase are soluble. Thus, the difference may lie in the requirement for different numbers of monocyte/macrophages for antigen presentation.

The observation that diminished T cell numbers and responses were associated with the presence of auto-antibodies may indicate that auto-immune mechanisms are involved in the pathogenesis of alopecia areata. Confirmation of an auto-immune mechanism requires the demonstration of reactivity against follicle constituents. Although reactivity could not be detected in lymphocytes it could have been masked by inhibitory substances the effects of which were demonstrable at higher concentrations. It may also be that relevant antigens were not present in adequate concentrations or that auto-reactivity may involve other lymphocytes than those that undergo blast transformation *in vitro*. Alternatively alopecia areata and impaired T lymphocyte function may both be manifestations of some other aetiological factor as in congenitally athymic nude mice and the tendency to develop auto-antibodies may therefore be secondary to diminished activity of regulatory mechanisms such as suppressor T cells.

Thus, although the present findings demonstrate a close association between decreased T cell reactivity auto-antibodies and alopecia areata, the nature of the relationship between the immune abnormalities and the hair loss is not clear.

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Alopecia areata and auto-immunity

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SUMMARY

The prevalence of auto-antibodies against thyroid constituents, gastric parietal cells, smooth muscle cells, mitochondria, reticulin, nuclear constituents and rheumatoid factor in 108 patients with alopecia areata was compared with that found in a previous survey of the local population. Female patients had a significantly increased prevalence of anti-thyroid antibodies which were present in 30% overall and in 44% of the youngest age group (11–17 years). Smooth muscle antibodies were more frequent in female patients but the increase was not significant. Male patients had a significantly increased prevalence of thyroid and gastric parietal cell antibodies (11·4% each). In females, antithyroid antibodies were associated with extensive hair loss: they were found in 42% of female patients with total alopecia and only 20% of males with total hair loss.

A family history of alopecia areata was obtained from 24% of patients; 10% had relatives with thyroid disease and 10% had diabetic relatives. These findings confirm the association between alopecia areata and the other auto-immune diseases.

Circumstantial evidence indicates that alopecia areata may be an auto-immune disease. The most direct evidence is provided by the histological finding that in the early stages the lower ends of hair follicles are surrounded by an infiltrate of lymphocytes. In addition, the hair loss, particularly in the early stages, may be reversed by corticosteroids given either locally or systemically. Many of the organ-specific auto-immune diseases occur in association with each other and they also occur with increased frequency in patients with alopecia areata and their relatives (Muller & Winkelmann, 1963; Cunliffe *et al.*, 1969; Main *et al.*, 1975). Immunological markers in the form of auto-antibodies, particularly against thyroid constituents and gastric parietal cells, are formed with greatly increased frequency in patients with organ-specific auto-immune diseases (Irvine, 1974). There are conflicting observations concerning the occurrence of such antibodies in alopecia areata. Thus, several authors reported positive associations between alopecia areata and auto-antibodies (Kern *et al.*, 1973; du Vivier & Munro, 1975; Main *et al.*, 1975) while others reported a lack of such association (Cunliffe *et al.*, 1969; Betterle *et al.*, 1975; Cochran, Thomson & MacSween, 1976; Klaber & Munro, 1978). The present study formed part of an investigation of immune reactivity in alopecia areata and results are presented in order to resolve the controversy surrounding the prevalence of auto-antibodies in this disease.

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PATIENTS AND METHODS

Patients

One hundred and eight patients with alopecia areata who had been referred to the dermatology department were studied (Table 1). They were asked whether their relatives had alopecia areata, thyroid disease, diabetes, pernicious anaemia or vitiligo. The extent and activity of the alopecia were noted and they were examined for signs of other diseases.

TABLE 1. Distribution of sex, age and disease extent in patients with alopecia areata

	Female	Male
Number	73	35
Mean age ± s.d.	37 ± 15·8	33·1 ± 13·5
Total alopecia	24	15

Methods

Serum was examined by indirect immunofluorescence for the presence of antibodies to thyroid cytoplasm, gastric parietal cells, smooth muscle cells, mitochondria, reticulin and nuclear constituents. Antibodies against thyroid microsomes were detected by complement fixation (Roitt & Doniach, 1958). Thyroglobulin antibodies were detected by the tanned red cell method using formalized sheep erythrocytes (Burroughs Wellcome); titres of 1/25 or greater were considered as abnormal. Rheumatoid factor was detected by the Rose-Waaler test.

The tests were compared with figures obtained by the same laboratory in a previously published survey of 2,779 members of the local population (Tunbridge *et al.*, 1977).

RESULTS

Association with other diseases in patients and relatives

Four female patients had active thyroid disease and two had vitiligo (Table 2). One male patient had insulin-dependent diabetes and vitiligo. Twenty female patients (27%) and seven males (20%) gave a history of alopecia areata in members of their family (Table 2). Eleven patients had relatives with thyroid disease, eleven with diabetes, four with pernicious anaemia and one with vitiligo.

TABLE 2. Prevalence of other diseases in patients with alopecia areata and their relatives

	Female patients	Affected families	Male patients	Affected families
Alopecia areata	73	20 (27)	35	7 (20)
Thyroid disease	4 (5·5)	9 (12·3)	0	2 (5·7)
Diabetes	0	9 (12·3)	1 (2·9)	2 (5·7)
Pernicious anaemia	0	3 (4·1)	0	1 (2·9)
Vitiligo	2 (2·7)	1 (1·4)	1 (2·9)	0

Figures in brackets are percentages.

Prevalence of auto-antibodies

In patients with alopecia areata, antibodies against thyroid constituents were found significantly more frequently than in the normal population (Table 3) and the increase was greatest in females. In the normal female population, anti-thyroid antibodies were found more commonly with increasing age. This trend was also observed in females with alopecia over 18 years of age but in the youngest age group (11–17 years) four of nine (44%) had anti-thyroid antibodies (Table 4).

TABLE 3. Auto-antibodies in patients with alopecia areata and the normal population

	Females				Males			
	Patients		Controls*		Patients		Controls*	
	No.	%	No.	%	No.	%	No.	%
Total tested	73		1,494		35		1,285	
Antibody								
Thyroglobulin	8	11†		3·0	1	2·9		0·9
Thyroid cytoplasm	15	21‡		10·3	4	11·4‡		2·7
Thyroid microsomes	16	22†		7·6	4	11·4†		2·0
Thyroid (all three)	5	7			1	2·9		
Thyroid (any)	22	30†		11·2	4	11·4‡		3·1
Thyroid + other	5	7			2	5·7		
Gastric parietal	3§	4		7·8	4	11·4‡		4·9
Smooth muscle	3¶	4		1·6	0	0		1·6
Mitochondria	0	0		0·7	0	0		0
Reticulin	0	0		0·9	0	0		2·2
Anti-nuclear	3	4		4·9	1	2·9		2·2
Rheumatoid factor	2§	3		2·2	1	2·9		2·6

* = Control values from Tunbridge *et al.*, 1977.
† = $P < 0{\cdot}001$ compared with control population.
‡ = $P < 0.01$ compared with control population.
§ = All had thyroid antibody.
¶ = 1 had thyroid antibody.

TABLE 4. Age-specific prevalence of anti-thyroid antibodies in patients with alopecia areata and controls

Age	11–17	18–24	25–34	35–44	45–54	55–64	65–74	75+
Females:	9	7	15	10	14	12	1	0
with antibodies	4	1	4	4	6	3	0	0
%	44	14	27	40	43	25	0	0
% in controls*	ND	8·6	7·1	9·7	14·6	14·3	13·7	12·5
Males	4	7	8	12	3	0	1	0
with antibodies	0	0	0	2	2	0	0	0
%	0	0	0	17	66	0	0	0
% in controls	ND	1·8	3·4	2·7	3·1	2·9	4·6	3·6

* Control figures from a survey of the occurrence of thyroid disease and auto-antibodies in 1494 females and 1285 males from the local population (Tunbridge *et al.*, 1977).

ND = Not done.

Antibodies to gastric parietal cells were significantly more frequent in male patients whereas in females they were slightly less common than in the normal population. Moreover, when detected in women, such antibodies were found only in those who also had anti-thyroid antibodies. Antibodies against smooth muscle were more common in female patients than controls but the increase was not significant (Table 3).

Of twenty-four female patients with total alopecia, ten (42%) had anti-thyroid antibodies while only three of fifteen males (20%) with total hair loss had such antibodies.

DISCUSSION

The results presented here confirm that there is indeed an increased association of auto-antibodies against thyroid constituents and gastric parietal cells in patients with alopecia areata. The significance of the observed frequencies for these antibodies is greatly increased by the comparison with prevalences reported by the same laboratory for 2,779 members of the local population (Tunbridge *et al.*, 1977). Other workers have reported findings in smaller groups of patients: from twenty-four by Klaber & Munro (1978) to seventy-eight by Main *et al.* (1975). More importantly, however, in most studies control groups have scarcely been larger although Kern *et al.* (1973) had 554 controls and Cunliffe *et al.* (1969) alluded to data from 469 patients in a general medical practice but gave no details. Most other reports did not distinguish the findings in females from those in males in either patients or controls. The present observations and those of Tunbridge *et al.* (1977) for the normal local population show that the differences between the sexes must be taken into account. The differences between females and males are further emphasized by the apparent association between total alopecia and anti-thyroid antibodies in females. The figures did not attain significance but this probably reflects the small numbers. There were other interesting differences between males and females. Gastric parietal cell antibodies were significantly more frequent in male patients than in controls. The three female patients in whom they were found also had anti-thyroid antibodies. The youngest group of female patients had a striking increase in anti-thyroid antibodies and it may be that this is related to the poor prognosis that is described for early onset of alopecia (Walker & Rothman, 1950).

The association of organ-specific auto-immune diseases such as thyroid diseases and diabetes mellitus in families of patients with alopecia areata has been confirmed by all observers (Muller & Winkelmann, 1963; Cunliffe *et al.*, 1969; Main *et al.*, 1975; Kern *et al.*, 1973; Klaber & Munro, 1978). The familial predisposition to develop auto-immune diseases and the association with auto-antibodies in patients with alopecia areata is analogous to findings in other auto-immune diseases (Irvine, 1974) and strengthens the possibility that alopecia areata is itself an auto-immune disease. It is not clear, however, whether specific anti-follicle auto-reactivity occurs although antibodies have not been found by direct immunofluorescence in affected scalp (Kern *et al.*, 1973; Bystryn, Orentreich & Stengel, 1979; Igarashi, Takeuchi & Sato, 1980). Moreover, in myasthenia gravis and Graves' disease, the specific anti-receptor antibodies thought to be responsible for the clinical manifestations cannot be demonstrated by immunofluorescence. Clearly a harder look for anti-follicle reactivity in alopecia areata is necessary.

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Dermatitis herpetiformis: immune complex detection with C1q and monoclonal rheumatoid factor

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SUMMARY

To determine the significance of circulating immune complexes in dermatitis herpetiformis, serum samples from thirty patients with active disease were tested by a C1q binding radioassay, while serum samples from twenty-one of these patients were tested by a monoclonal rheumatoid factor (mRF) inhibition radioassay. By direct immunofluorescence, all patients demonstrated typical IgA deposition in dermal papillae. Using the C1q binding assay, only seven of forty-two serum samples had elevated C1q binding activity, while by the mRF inhibition assay, thirteen of twenty-five samples had elevated immune complex levels. Nine of these latter thirteen positive serum samples, however, were minimally elevated. Thus, IgG and/or IgM containing immune complexes are infrequently present, or at very low levels, in sera of patients with active dermatitis herpetiformis.

Dermatitis herpetiformis is a chronic progressive skin disease characterized by grouped vesicular lesions and intense pruritus. In addition, there is an increased incidence of gluten sensitivity enteropathy (Fry *et al.*, 1967) and the histocompatability antigens, HLA, B8 and Dw3 (Katz & Strober, 1978). Histopathologically, early skin lesions show papillary microabscesses of neutrophils and eosinophils with subepidermal vesiculation (MacVicar, Graham & Burgoon, 1963). The aetiology, however, remains unknown.

As in other blistering skin diseases, specific immunopathological findings have been identified in dermatitis herpetiformis; that is, IgA deposition in the tips of the dermal papillae (beneath the dermal-epidermal junction) has become a diagnostic hallmark of the disease (Fry & Seah, 1974; Katz & Strober, 1978). Such IgA deposition may be seen in both normal and perilesional skin,

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either during active disease or in normal skin of patients in clinical remission. Complement components, including C3, factor B and properdin, have been noted in perilesional skin, perhaps suggestive of alternative pathway activation (Provost & Tomasi, 1974); C1q and C4 are rarely seen (Seah *et al.*, 1973). The nature of these deposits, however, is still not clear.

Anticomplementary activity, suggestive of immune complex formation, was initially identified by Mowbray *et al.* (1973) in sera of twelve of fifteen patients with active dermatitis herpetiformis. Extending those studies, Mohammed *et al.* (1976) detected circulating immune complexes in 100% of serum samples from fifty-nine patients with dermatitis herpetiformis using three methods: (1) a radiobioassay, (2) a C1q binding radioassay, and (3) polyethylene glycol precipitation. The present study was undertaken to: (1) confirm the presence and incidence of immune complexes in dermatitis herpetiformis using a C1q binding radioassay (Tappeiner *et al.*, 1977) and a monoclonal rheumatoid factor (mRF) inhibition radioassay (Luthra *et al.*, 1975), and (2) correlate such findings with immunoglobulin (other than IgA) and complement deposition in dermatitis herpetiformis skin.

MATERIALS AND METHODS

Reagents utilized in these studies included: Sepharose 6B, Sephadex G-25 and Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden); IgG Cohn Fraction II (Research Products Division, Miles Laboratories, Inc. Elkhart, Indiana, U.S.A.); disodium ethylenediaminetetraacetate (EDTA) (Fisher Scientific Co., Pittsburgh, Pennsylvania, U.S.A.); CnBr (Eastman Kodak Co., Rochester, New York, U.S.A.); polyethylene glycol (PEG) 6000, agarose, lactoperoxidase (Sigma Chemical Co., St. Louis, Missouri, U.S.A.); avicel microcrystalline cellulose (Brinkman Instruments, Inc., Westbury, New York, U.S.A.); and ^{125}I (New England Nuclear, Boston, Massachusetts, U.S.A.).

All thirty patients included in this study had active skin lesions when serum samples were collected. In all instances, the histopathological pattern was compatible with dermatitis herpetiformis, and all patients were responsive to conventional sulphapyridine or sulphone therapy.

Direct IF staining was performed by previously detailed methods (Jordon, 1976) using monospecific antisera to IgG, IgA, IgM and C3 (Meloy Laboratories, Inc., Springfield, Virginia, U.S.A.). Antisera were tested for activity and specificity by immunoelectrophoresis and double immunodiffusion. Use of the various labelled antisera conformed to previously determined standards (Beutner, Chorzelski & Jordon, 1970). In order to be included in the study, all patients had to demonstrate typical granular IgA deposition in the dermal papillae (Fig. 1).

For immune complex detection, two radioimmunoassays were utilized employing our previously published methods (Mackel *et al.*, 1979). One assay, a C1q binding radioassay, involved the interaction of immune complexes with C1q component of complement. This assay therefore detects complexes of sufficient size for complement activation (Lightfoot, Drusin & Christian, 1970). The second assay, an mRF inhibition radioassay, involves the interaction of IgG containing complexes with mRF. In addition to detecting large IgG containing immune complexes, this assay also detects complexes of smaller size or those not capable of detection by complement binding assays (Mackel *et al.*, 1979).

IgG (25 mg/ml) was heat aggregated at 63°C for 12 min, separated by gel filtration on Sepharose 6B in 0·1 M Tris-HCl, 0·2 M NaCl, pH 8·5; only the protein eluting in the exclusion volume was utilized as heat aggregated (Agg) IgG (Tappeiner *et al.*, 1977). Agg IgG served as a standard for both radioassays

The C1q binding radioassay, originally described by Nydegger *et al* (1974), was performed with modifications outlined by Zubler *et al.* (1976) and Tappeiner *et al.* (1977). C1q was isolated by the method of Yonamasu & Stroud (1971) and labelled with ^{125}I in a lactoperoxidase catalysed reaction

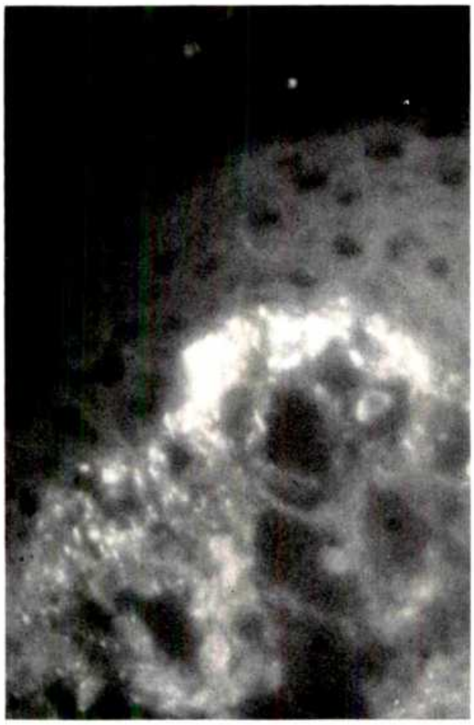

FIGURE 1. Direct immunofluorescence of normal skin from a patient with dermatitis herpetiformis using labelled antiserum to human IgA. Typical granular deposition of IgA is apparent in the dermal papilla (× 400).

(Heusser *et al.*, 1973). Aliquots of serum (0·2 ml) were diluted with an equal volume of 0·26 M EDTA pH 7·5, and 0·5 ml of a 5 μg/ml solution of ^{125}I-C1q was added to each tube. Following a 1-h incubation at room temperature, 3 ml of PEG 6000 solution was added to a final concentration of 1·5% PEG. Tubes were then incubated at 4°C, centrifuged, the supernates discarded, and the remaining activity counted in a gamma scintillation spectrometer (Packard Instruments Co., Downers Grove, Illinois, U.S.A.). The radioactivity remaining in the precipitate was expressed as percent of the total [^{125}I] C1q activity and compared to binding of known concentrations of Agg IgG (1–1000 μg). Levels of immune complexes in each serum tested or C1q binding activity (C1qBA) were expressed in terms of μg equivalent Agg IgG per ml of serum (μg Agg Eq/ml); 5 μg Agg Eq/ml or less C1qBA was considered normal.

A solid-phase mRF inhibition assay was performed by the method of Luthra *et al.* (1975). mRF was purified from macroglobulinaemic serum by dialysis against 0·1 glycine HCl, pH 3·0 for 12 h at 4°C, followed by Sephadex G-200 column chromatography using the same buffer. The exclusion peak was coupled to microcrystalline cellulose beads with CnBr. Aliquots (0·5 ml) of serum (1:10 dilution), 0·5 ml ^{125}I-labelled Agg IgG, and 0·5 ml mRF cellulose were incubated in tubes, centrifuged, washed with buffer and again counted in a gamma scintillation counter. As with the C1q binding assay, results were again compared to a standard curve of Agg IgG (0·3–4 μg/ml) in normal human serum, and again expressed as μg Agg IgG per ml. Levels greater than 5 μg Agg Eq/ml were considered abnormal.

RESULTS

Forty-two serum samples from thirty clinically active dermatitis herpetiformis patients were tested for the presence of circulating immune complexes by the C1q binding radioassay. Only seven serum samples (17%) from six patients had elevated C1qBA (Fig. 2); one of the positive serum samples was minimally elevated (6 Agg Eq/ml). One serum sample, however, had very high C1qBA at 100 μg Agg Eq/ml.

By the mRF inhibition radioassay, twenty-five serum samples from twenty-one dermatitis herpetiformis patients were tested for immune complexes. Elevated levels were present in thirteen of the twenty-five samples (52%), but nine of the samples (Fig. 2) were again only minimally elevated (< 10 μg Agg Eq/ml). Three of twenty-five serum samples tested by the two radioassays demonstrated both elevated C1q and mRF reactivity.

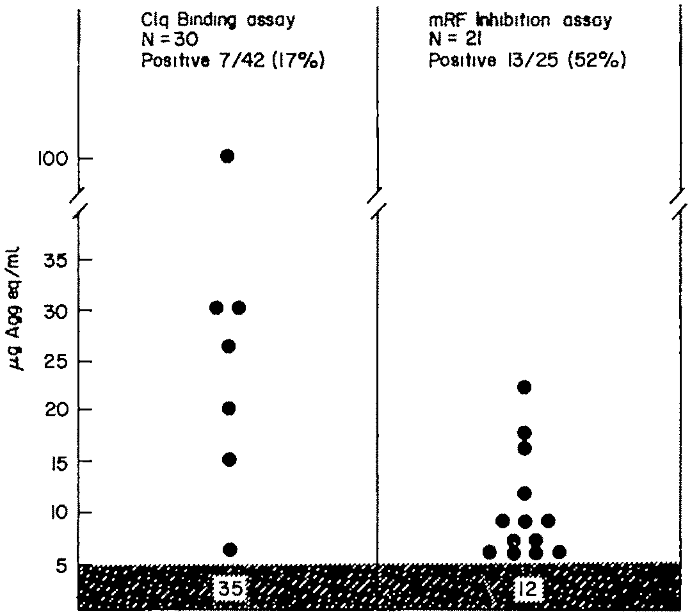

FIGURE 2. Scattergram demonstrating Clq and mRF binding activity in serum samples of patients with dermatitis herpetiformis. Hatched areas indicate normal levels (less than 5 μg Agg Eq/ml).

Table 1 summarizes direct IF findings and immune complex detection results in the twenty-one patients tested by both radioassays. Ten of the twenty-one patients studied had IgA deposition alone in the dermal papillae; that is, in the absence of other immunoglobulins and C3. Six of thirteen serum samples from patients with IgA deposition alone were positive by the mRF inhibition assay; all thirteen serum samples, however, were negative by the C1q binding assay. Five of the twenty-one patients had concomitant C3 deposition in addition to IgA. Two of five serum samples in this group had elevated mRF reactivity but again negative C1qBA. In addition to both IgA and C3, six of the twenty-one patients had either IgG or IgM (or both) deposited in dermal papillae. Interestingly, five of six serum samples in this group were positive by the mRF inhibition assay, and three of six were positive by the C1q binding assay. In addition, the latter three serum samples had immune complexes detectable by both radioassays.

TABLE 1. Summary of direct immunofluorescence (DIF) findings and detection of circulating immune complexes in twenty-one patients with dermatitis herpetiformis

DIF findings in skin*	mRF	C1q	Both
IgA alone (ten patients)	6/13	0/13	0/13
IgA + C3 (five patients)	2/5	0/5	0/5
IgA + IgG and/or IgM + C3 (six patients)	5/6	3/6	3/6

* Granular deposition primarily in the dermal papillae of normal skin.
mRF = Monoclonal rheumatoid factor assay; C1q = C1q binding assay.

As in our previously reported studies (Tappeiner *et al.*, 1977; Mackel *et al.*, 1979), all serum samples from normal individuals have been consistently negative (<5 μg Agg Eq/ml) when tested by both radioassays.

DISCUSSION

By the C1q binding radioassay, only seven of forty-two serum samples (17%) from thirty dermatitis herpetiformis patients had elevated C1qBA. These findings therefore contrast significantly with those reported by Mohammed *et al.* (1976) who, using a similar C1q binding radioassay, reported detectable immune complexes in seventy of eighty-three serum samples (83%) from fifty dermatitis herpetiformis patients. Our findings, however, are similar to those recently summarized by Hall *et al.* (1980) who demonstrated elevated C1qBA in only three of forty-seven (6%) patients tested. Further, these same investigators detected IgG containing immune complexes in only three of forty-six (7%) serum samples tested by the Raji cell radioassay.

Approximately 50% of the dermatitis herpetiformis serum samples tested were positive by the mRF inhibition assay. As mentioned previously, the mRF assay is capable of detecting IgG containing complexes of much smaller size than the C1q binding assay (Mackel *et al.*, 1979); that is, the mRF assay will detect immune complexes that are not large enough to activate complement. Perhaps in dermatitis herpetiformis immune complexes form, which for the most part are small and not detectable by complement-dependent assays (C1q binding and Raji cell assays), but may be detected by the mRF assay. Nine of the thirteen mRF-positive sera, however, were only minimally elevated and of questionable significance.

Although the number of patients studied was too small to draw specific conclusions, the presence of immune complexes detectable by C1q binding correlated with the presence of immunoglobulins (IgG and/or IgM) other than IgA in dermatitis herpetiformis skin. These same three serum samples were also reactive in the mRF assay. The mRF assay, however, was frequently positive in serum samples of patients with IgA deposition alone.

Since IgA deposition in dermal papillae has become an IF hallmark of this disease process, recent investigations have focused upon detection of immune complexes containing IgA. Using a modification of the Raji cell radioassay, Zone, LaSalle & Provost (1980a) reported IgA containing immune complexes in six of eighteen patients with dermatitis herpetiformis. Levels of these immune complexes, however, tended to be low when compared to normals.

More recently, these same investigators have suggested that these complexes contain gluten (Zone, LaSalle & Provost, 1980b). Hall *et al.* (1980), however, found IgA containing complexes in only twelve of forty-nine dermatitis herpetiformis serum samples. Again, the levels tended to be low, and no correlation between levels of IgA complexes and disease activity was apparent in their study.

Thus, the role (if any) of IgA immune complexes in the pathogenesis of dermatitis herpetiformis is not resolved.

In contrast to findings reported earlier by Mohammed *et al.* (1976), IgG and/or IgM containing immune complexes are not frequently present in sera of patients with dermatitis herpetiformis. When present, they are at relatively low levels and are usually of a size not capable of complement activation. They are most likely epiphenomenal rather than pathogenetic. Although IgA containing immune complexes may be important, mechanisms other than immune complex formation and deposition should be considered in the pathogenesis of this disease.

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Inflammatory cells, IgA, C_3, fibrin and fibronectin in skin lesions in dermatitis herpetiformis

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SUMMARY

Skin lesions were produced by application of 50% potassium iodide to twelve patients with dermatitis herpetiformis (DH). Perivascular cellular infiltrates were found to be characteristic of developing lesions. The cells were mainly round cells; α-naphthyl acetate esterase staining revealed that in 24-h lesions the mean percentage of T-lymphocytes was 43%, that of mononuclear phagocytes 6% and that of non-T/non-M cells (mainly B-lymphocytes) 44%. The percentage of the latter was highest (mean 81%) in 6-h specimens, suggesting that these cells are participating in the early stages of lesion formation. The infiltrating cells in dermal papillae and within subepidermal vesicles were predominantly polymorphonuclear leukocytes (mean 86%) with some mononuclear phagocytes and non-T/non-M cells.

Immunofluorescence examination confirmed that fibrin deposition is characteristic of the initial lesions of DH and showed that the same is true of fibronectin. Seven out of eight patients had fibronectin deposits in dermal papillae. IgA was found in all and C_3 in most of the specimens and, with the exception of papillary vesicles and blister cavities, the intensity of IgA and C_3 fluorescence showed no marked alterations during the development of lesions.

The skin lesion in dermatitis herpetiformis (DH) is characterized by subepidermal vesicles, polymorphonuclear leukocyte (PMN) micro-abscesses and deposits of IgA, the third component of complement (C_3) and fibrin in the papillary dermis. A frequently proposed hypothesis postulates that IgA originates in the form of immune complexes from the small intestine and binds to the skin, where it activates complement with subsequent release of chemotactic factors and resultant influx of PMN (van der Meer, 1972; Seah *et al.*, 1973; Katz & Strober, 1978). However, the antigen to these tissue bound IgA antibodies remains unclear and additional factors seem to be important in producing visible skin lesions because both IgA and C_3 are frequently found in the normal-appearing skin of patients with DH (van der Meer, 1972; Katz *et al.*, 1976; Haffenden, Wojnarowska & Fry, 1979).

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In this study skin lesions were induced by local application of potassium iodide (KI) to patients with DH. The mechanism of action of KI is unknown but it elicits lesions similar to natural lesions and enables the exact ageing of these lesions (Salo *et al.*, 1970; Alexander, 1975; Katz & Strober, 1978). The cellular infiltrates in developing lesions were analysed by quantifying T lymphocytes, mononuclear phagocytes and PMN in order to study the role these cells may play in lesion formation. Moreover, the lesions were examined with immunofluorescence (IF) for IgA, C_3, fibrin and fibronectin (FN). The latter is an important agent mediating cell-to-cell and cell-to-matrix attachment and deviations from its normal skin distribution have been recently found in lichen planus, lupus erythematosus and systemic sclerosis (Yamada & Olden, 1978; Cooper *et al.*, 1979; Fyrand, 1979b, 1980).

PATIENTS AND METHODS

Patients

Twelve patients, seven males and five females, with DH were examined. The diagnosis of DH was based on a typical clinical picture and histopathology of the rash and on the demonstration of granular IgA deposits in the papillary dermis. All patients were taking dapsone (50–150 mg/day) which was stopped at least 3 days before the examination.

Potassium iodide application

Skin lesions were produced by applying chambers containing 50% KI in petrolatum on the middle back of the patients as described previously (Förström *et al.*, 1979). The chambers were removed after 24 h and the lesions were graded macroscopically as many small papulovesicles (grade III), some papulovesicles (grade II), erythema and/or oedema without vesicles (grade I) or no visible lesions. Chambers with only petrolatum served as controls in every patient.

Biopsies were taken from 24-h skin lesions and from control (petrolatum) skin areas of nine patients. Biopsies were also taken from three patients at 0, 6, 12 and 24 h after KI application. One natural lesion, less than 24 h old, was biopsied from one of the patients. All biopsy specimens were cut immediately into three pieces; two were processed for histochemical staining and the third was snap frozen for IF examinations.

Histochemical methods

Biopsy specimens were processed for the demonstration of α-naphthyl acetate esterase (ANAE) staining in T lymphocytes and mononuclear phagocytes (termed here macrophages) as described in detail earlier (Ranki *et al.*, 1980). Briefly, the tissues were fixed in Baker's formol calcium for 20–24 h at 4°C, washed in Holt's buffer, dehydrated in acetone and cleared in xylene, all at 4°C, before paraffin embedding at 56°C. Blocks were sectioned at 6 μm and two consecutive sections were always prepared, one for ANAE and one for routine H & E staining. ANAE staining differentiates T lymphocytes (dot-like staining) from macrophages (diffuse cytoplasmic staining). All the unstained mononuclear cells were termed non-T/non-M cells. PMN were identified by morphology in H & E sections. The various cells were counted using an ocular counting square under ×400 magnification as described earlier (Reitamo *et al.*, 1981). When present, cellular infiltrates were counted in three distinct areas, i.e. in the perivascular areas of mid and upper dermis, in the dermal papillae and within subepidermal vesicles. At least 150 cells were counted from each area and the final counts were expressed as a percentage of the total cell count.

Immunofluorescent methods

Skin biopsy specimens for IF were stored at -50°C until stained. They were examined simultaneously for IgA, C_3 and fibrin using commercial (Behringwerke) FITC-conjugated antisera diluted to 1/20 as described earlier (Reunala, 1978). FN was examined using rabbit antihuman FN antisera (Vuento & Vaheri, 1979) diluted to 1/50 at the first step and commercial FITC-conjugated goat anti-rabbit antisera diluted to 1/20 at the second step. At least five tissue sections were examined by two observers and the quantity of IgA, C_3, fibrin and FN was graded by the amount of fluorescent material in the papillary dermis as strong (+ + +), moderate (+ +), weak (+) and no (−) fluorescence.

RESULTS

Potassium iodide induced macroscopic lesions in all patients (Fig. 1). Six of the patients had grade I or II lesions and the rest grade III lesions. None of the patients had macroscopic lesions in the control skin area.

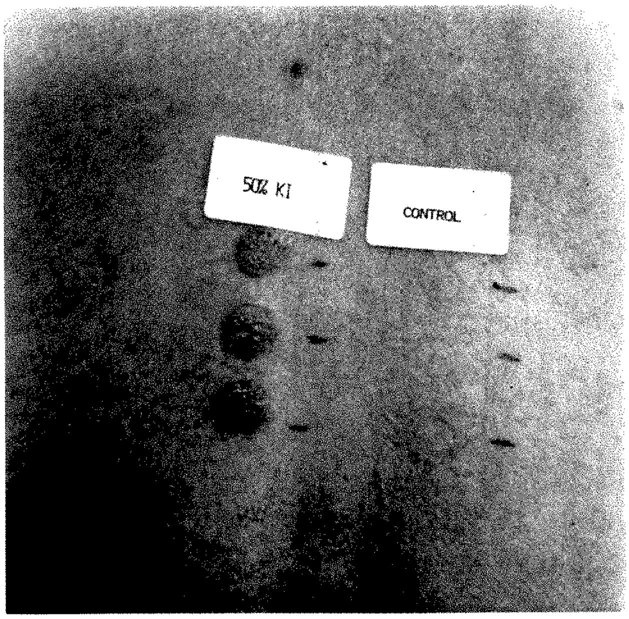

FIGURE 1. Skin lesions produced by 24-h application of potassium iodide in a patient with dermatitis herpetiformis.

Inflammatory cells

All patients showed perivascular cellular infiltrates in 24-h specimens (Fig. 2). The cells were predominantly round cells. The mean percentage of non-T/non-M cells was 44%, that of T lymphocytes 43% and that of macrophages 6%. The variation between the percentages of various round cells was rather large but PMN were found only in perivascular infiltrates of grade III lesions (mean 13%) whereas their amount was neglible (< 1%) in grade I and II lesions (Table 1). Papillary and intravesicular infiltrates were typical of grade III lesions, and the cells were mainly PMN (mean 86%) with some macrophages (mean 12%) and an occasional non-T/non-M cell. Because there were few or no papillary infiltrates and vesicles in grade I and II lesions, mean cell counts were not available for the whole patient group. A natural lesion showed infiltrates similar to those found in KI-induced

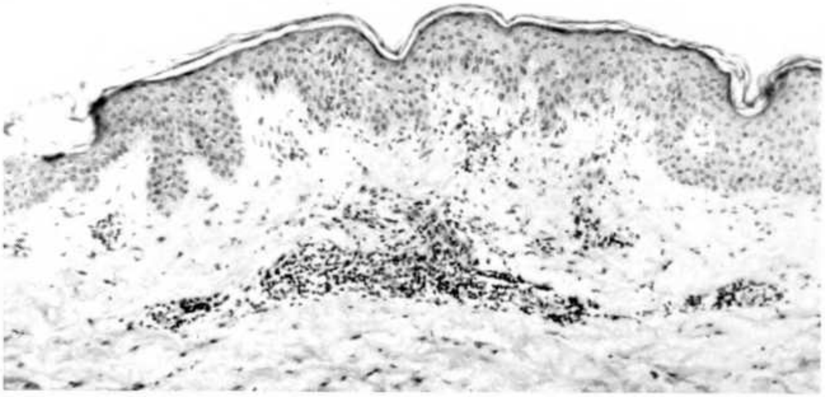

FIGURE 2. Perivascular cellular infiltrate in a weak DH skin lesion produced by 24-h application of potassium iodide (H & E staining, × 80).

lesions in the same patient. Perivascular infiltrates of natural lesion consisted of non-T/non-M cells (68%), T lymphocytes (22%) and macrophages (8%) and papillary and intravesicular infiltrates were predominantly PMN (93%). Control skin specimens showed no cellular infiltrates.

The cellular infiltrates were absent in 0-h specimens but all three patients showed perivascular infiltrates in 6, 12 and 24-h specimens (Table 2). The percentage of non-T/non-M cells decreased and that of T lymphocytes increased somewhat during the development of skin lesions. The appearance of PMN correlated again with the macroscopic findings. The patient with the grade III 24-h lesions showed PMN in perivascular and papillary infiltrates of the 6-h specimen, and intravesicular PMN (99%) were seen in the 12-h specimen. In the patient with grade II lesions the changes developed later; PMN were seen perivascularly in the 12-h specimen and in papillae in the 24-h specimen. The patient with the weakest (grade I) lesions did not show PMN in any areas at all.

Immunofluorescence findings

Every patient had IgA deposits both in lesional and normal-appearing control skin (Table 3). The intensity of IgA fluorescence in these areas was the same in six patients and differed by one grading in three patients. C_3 deposits occurred in the lesional and control skin in seven patients, one had C_3 only in the lesional skin and one did not show C_3 at all. The only sites where IgA and C_3 could be absent were the areas of fully developed vesicles and blister cavities of grade III lesions (Fig. 3). However, both IgA and C_3 were always present in the perivesicular areas of the same tissue specimen. Fibrin and FN occurred in lesional skin (Fig. 4) but one patient each showed fibrin or FN in the papillary dermis of normal-appearing skin. A natural lesion showed IF findings similar to the KI-induced lesion of the same patient. The results were IgA ++/++, C_3 +/+, fibrin +++/+++ and FN +++/++, respectively.

The intensity of IgA and C_3 fluorescence remained fairly constant during the development of skin lesions (Table 4). The only exception was the disappearance of IgA and C_3 from the vesicular areas of the 24-h specimen of the patient with the grade III lesions. In contrast, fibrin and FN appeared

TABLE 1. Inflammatory cells in skin lesions produced by 24-h application of 50% potassium iodide in twelve patients with DH

	Perivascular infiltrates				Papillary/intravesicular infiltrates			
Degree of skin lesions	T lymphocytes* (%)	Macrophages* (%)	Non-T/non-M cells* (%)	Polymorphonuclears (%)	T lymphocytes* (%)	Macrophages* (%)	Non-T/non-M cells* (%)	Polymorphonuclears (%)
Patients with grade I or II lesions (n = 6)	53·0±23†	6·0±6	40·3±22	<1·0	not present‡			
Patients with grade III lesions (n = 6)	32·4±18	7·2±4	47·2±17	12·7±12	<1·0	11·6±10	2·8±3	85·6±10

* Stained with ANAE.
† Mean ± s.d.
‡ Present in only two patients.

TABLE 2. Perivascular inflammatory cells in skin lesions produced by 0, 6, 12 and 24-h application of 50% potassium iodide in three patients with DH

	T lymphocytes (%)				Macrophages (%)				Non-T/non-M cells (%)				Polymorphonuclears (%)			
Patients	0	6	12	24	0	6	12	24	0	6	12	24	0	6	12	24
Pat. A.L. (grade I lesion)	0*	6	27	34	0	<1	5	1	0	94	68	65	0	<1	<1	<1
Pat. J.L. (grade II lesion)	0	10	21	28	0	10	9	13	0	80	66	52	0	<1	4	7
Pat. P.O. (grade III lesion)	0	20	25	34	0	5	4	<1	0	70	22	32	0	5	49	34

*0 = No analysable infiltrates present.

TABLE 3. Immunofluorescence findings in skin lesions produced by 24-h application of 50% potassium iodide in nine patients with DH

	IgA				C_3				Fibrin				Fibronectin†			
	+++*	++	+	−	+++	++	+	−	+++	++	+	−	+++	++	+	−
Lesional skin (n = 9)	4	4	1	0	2	5	1	1	5	1	1	2	0	4	3	1
Normal-appearing control skin (n = 9)	3	5	1	0	0	2	5	2	0	1	0	8	0	1	0	7

* Degree of fluorescence in papillary dermis.
† Examined in only eight patients.

TABLE 4. Immunofluorescence findings in skin lesions produced by 0, 6, 12 and 24-h application of 50% potassium iodide in three patients with DH

	IgA				C_3				Fibrin				Fibronectin			
	0	6	12	24	0	6	12	24	0	6	12	24	0	6	12	24
Pat. A.L. (grade 1 lesion)	+++*	+++	+++	+++	++	++	++	+++	−	−	++	+++	−	+	+	++
Pat. J.L. (grade II lesion)	++	++	++	+	+	+	++	+	−	−	++	++	−	+	+	++
Pat. P.O. (grade III lesion)	++	+++	+++	+++	−	++	+	−	−	+	++	++	nd	nd	++	nd

* Degree of fluorescence in papillary dermis.

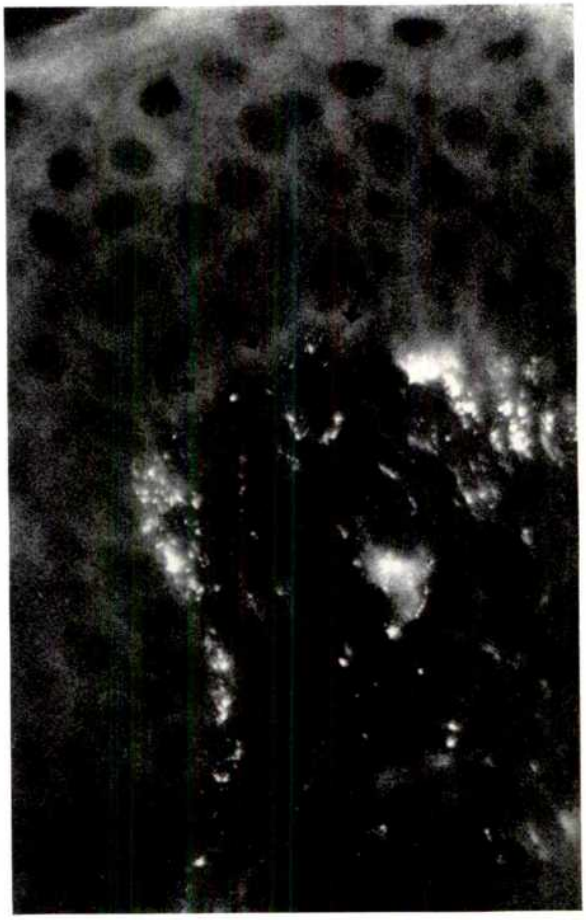

FIGURE 3. Incipient disappearance of IgA from the tip of a dermal papilla (arrows) in an early stage of vesicle formation (direct IF, × 420).

TABLE 5. Summary of cellular and IF findings during development of 50% potassium iodide induced skin lesions in three patients with DH

	Cellular infiltrates in dermis			IF findings in papillary dermis			
	Perivascular (mainly round cells)	Papillary (mainly PMN)	Intravesicular (mainly PMN)	IgA	C_3	Fibrin	Fibronectin
Initial stage	o*	o	o	+	+	o	o
6-h lesion	+	±	o	+	+	±	+
12-h lesion	+	±	±	+	+	+	+
24-h lesion	+	+	±	+	+	+	+

*o = Not present; ± = present in one; + = present in two or all three patients.

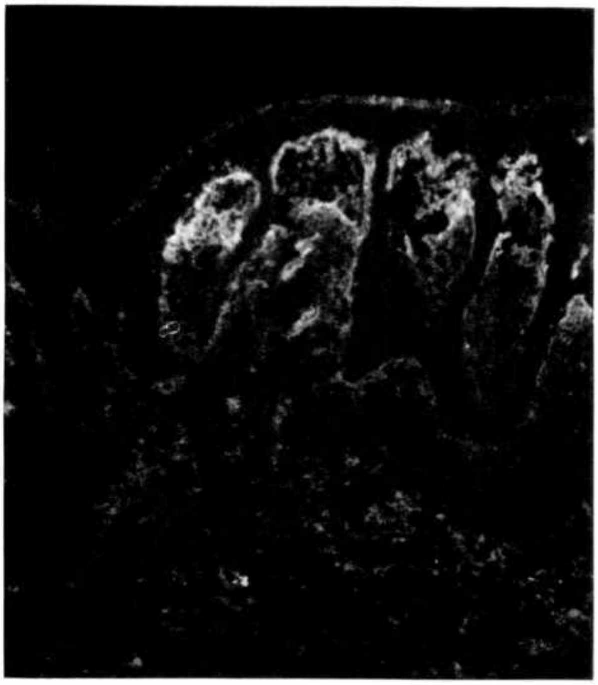

FIGURE 4. Fibronectin deposits in the dermal papillae of a 24-h DH lesion (indirect IF, × 125).

during the development of lesions and their fluorescence seemed to be most intense in 24-h specimens. The patient with the grade I lesions showed fibrin and FN in the papillary dermis although PMN were not seen in any areas at all. The patient with grade II lesions showed fibrin and FN before PMN appeared in dermal papillae and vesicles were formed. Table 5 summarizes the cellular and IF findings during the development of skin lesions in the present three patients.

DISCUSSION

The typical histological picture of DH was determined when attention was focused on the early stages of blister formation rather than on the fully developed subepidermal bulla. According to Pierard & Whimster (1961) the initial lesion can be seen at some distance from the blister and consists of a small collection of PMN invading the tips of the dermal papillae with a subsequent increase in microabscesses and vesicles. The present study showed that perivascular round cell infiltrates are also characteristic of skin lesions of DH. Such infiltrates occurred in mid and upper dermis and were found in 6, 12 and 24-h lesions, i.e. even before PMN had appeared in the dermal papillae and before any vesicles had formed. Therefore, perivascular infiltrates are not secondary to blister formation and because they also were found in a natural lesion their occurrence is not due to the application of KI. Some earlier reports mention perivascular infiltrates in DH skin lesions. MacVicar, Graham & Burgoon (1963) noted that largely perivascular, and predominantly lymphocytic inflammatory infiltrates were present at the time the pathological changes were first seen in the dermal papillae.

Kresbach & Hartwagner (1968) and Katz & Strober (1978) mentioned that upper and mid-dermal vessels of early lesions are surrounded by a lymphohistiocytic infiltrate as well as some PMN.

The ANAE method used in this study permitted a more exact identification of the perivascular infiltrates. The cells were mainly non-T/non-M cells and T lymphocytes; macrophages were seen in smaller numbers and PMN were found only in perivascular infiltrates of grade III lesions. It has been shown that the cells not identified with ANAE, i.e. the non-T/non-M cells in this study, belong mainly to the B cell line (Ranki, Tötterman & Häyry, 1976). The percentage of these cells was highest in 6-h lesions after which their relative proportion decreased, whereas the proportion of T lymphocytes increased. However, the figures for non-T/non-M cells remained higher and those of T cells lower than the figures found by Braathen, Förre & Natvig (1979) in the peripheral blood of normal controls and patients with DH. Therefore, the high numbers of non-T/non-M cells (mainly B-lymphocytes) found in the developing lesions in the present study suggest that these cells are participating in the initial events of lesion formation. Some non-T/non-M cells also occurred in the dermal papillae and such cells may cause local production of immunoglobulins, as suggested by earlier observations made in DH skin by Lai A Fat, Cormane and van Furth (1974).

IF examinations have shown the importance of demonstrating IgA in the papillary dermis in the diagnosis of DH (van der Meer, 1972; Fry & Seah, 1974). However, one essential and as yet unanswered question in DH is, why the skin lesions occur mainly on elbows, knees and buttocks and not in other sites since both IgA and C_3 are also frequently found in normal-appearing skin of the patients. In this study the lesions were produced on the middle back, a site where only a few of the present patients had earlier had skin lesions. Nevertheless, IgA was present in every specimen taken from lesional and normal-appearing skin of the patients, the intensity of IgA fluorescence was not different between these areas and no marked alteration occurred in IgA fluorescence during the development of lesions. The only sites where both IgA and C_3 could disappear were the papillary vesicles and blister cavities in grade III lesions, which is in agreement with earlier findings (van der Meer, 1972). In this study C_3 fluorescence seemed to be more intense in lesional skin than in normal-appearing skin, but again, no marked alterations in C_3 fluorescence were found during the development of lesions. However, earlier studies have reported a decrease in C_3 in normal-appearing skin after gluten withdrawal, which suggests that complement factors might be involved in the genesis of skin lesions in DH (Fry *et al.*, 1978; Reunala, 1978).

Papillary deposition of fibrin is characteristic of the initial lesions of DH and fibrin deposits can occur even before micro-abscesses are fully developed (Mustakallio, Blomqvist & Laiho, 1970). The present findings agree with this and, moreover, showed that FN deposition occurs in the same way. FN occurred in dermal papillae and within the vesicles in a homogenous pattern well differentiated from its normal distribution in the dermo-epidermal junction and in neural and vascular structures of the skin (Fyrand, 1979a). FN fluorescence was most intense within the subepidermal vesicles in this study suggesting that it may be acting as an opsonizing agent for macrophages in the removal of fibrin and denatured collagen (Jileck & Hörmann, 1978). The occurrence of fibrin and FN in the papillary dermis of otherwise normal skin in one of the present patients suggests an inflammatory process, although one not capable of producing macroscopic or microscopic lesions. The increased prostaglandin activity found in the normal-appearing skin of patients with DH is in agreement with this finding (Förström *et al.*, 1979).

In conclusion, this study demonstrated that perivascular infiltrates, which consist of high numbers of non-T/non-M cells (mainly B-lymphocytes) with a smaller number of T-lymphocytes, are characteristic of the early stages of the developing skin lesions in DH. With the exception of papillary vesicles, IF examinations failed to reveal any marked alterations in the intensity of IgA and C_3 fluorescence but showed that deposition of fibrin and fibronectin was typical of developing lesions.

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Detection of immune complexes in systemic sclerosis and Raynaud's phenomenon

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SUMMARY

Phagocytosed immune complexes in polymorphonuclear leukocytes of nineteen patients with Raynaud's phenomenon were detected by scoring the cells for IgG and C3 inclusions. Parallel estimations of circulating immune complexes were made by liquid phase C1q binding and polyethylene glycol (PEG) precipitation methods. The results were compared with the degree of cold stress-induced vasospasm measured by quantified infra-red thermography. Thirteen of nineteen patients with Raynaud's phenomenon compared with only one of seventeen normal controls showed significantly elevated levels of phagocytosed IgG. The correlation of IgG and C3 scores for individual patients was high and the IgG and C3 scores correlated well with the thermographic assessment of the severity of the Raynaud's phenomenon. Two of the patients had elevated levels of circulating immune complexes detectable by raised levels of C1q binding, four patients had raised IgG and three patients raised IgM complexes detectable by PEG precipitation. None of the controls had elevated levels of circulating immune complexes detectable by either method. There was no correlation between the levels of circulating and phagocytosed immune complexes.

The aetiology of Raynaud's phenomenon remains obscure. It is a feature of over 80% of patients with systemic sclerosis and mixed connective tissue disease and is a well recognized finding in systemic lupus erythematosus. Many patients with Raynaud's phenomenon have no clinical stigmata of a connective tissue disease at first presentation but may show immunological abnormalities.

Experimental studies in animal models have shown that immune complexes may play an important role in the pathogenesis of vascular lesions leading to disease symptoms (Cochrane, 1968).

Van der Meulen *et al.* (1979) have recently reported the presence of immune complexes in peripheral blood polymorphonuclear leukocytes of patients with Raynaud's phenomenon and have correlated the level of vasospasm of the fingers due to cold provocation as measured by photoelectric plethysmography with the level of phagocytosed immune complexes.

The object of our study was to confirm the reported observation of intracellular immune complexes in polymorphonuclear leukocytes of patients with Raynaud's phenomenon and systemic sclerosis,

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and patients with Raynaud's phenomenon and immunological abnormalities suggestive of associated connective tissue disease but without good clinical evidence of systemic sclerosis or systemic lupus erythematosus. We also set out to evaluate the usefulness of the polymorphonuclear leukocyte immune complex score as an indication of impending connective tissue disease in the latter group of patients. Furthermore we attempted to correlate the polymorphonuclear leukocyte immune complex score with circulating levels of immune complexes and with the thermographic index, an objective measure of the response of the vasculature of the hands to cold stress measured by quantified infra-red thermography (Bowcock & Cooke, 1981; Cooke & Bowcock, 1980).

MATERIALS AND METHODS

A sample of venous blood was obtained from thirteen patients with Raynaud's phenomenon and systemic sclerosis, six patients with Raynaud's phenomenon suspected to be secondary to a connective tissue disease, i.e. having no clinical stigmata of a connective tissue disease but an immunological abnormality, e.g. positive antinuclear factor at low titre, and fifteen normal controls. Polymorphonuclear leukocytes were isolated by differential centrifugation of leukocytes from heparinized venous blood after prior lysis of erythrocytes with buffered 0·84% ammonium chloride. Cytocentrifuge slides of these cells were then air dried and fixed in acetone. Intracellular immunoglobulin and complement were detected by means of a double layer immunofluorescence technique using sheep anti-human IgG (Wellcome Laboratories, Beckenham, Kent) and rabbit anti-human C3 (Nordic Laboratories, Maidenhead, Berks), the antiserum being used at a dilution of 1 : 20 in bovine serum albumin, and rabbit anti-sheep and sheep anti-rabbit fluorescent isothiocyanate conjugates (Wellcome Laboratories, Beckenham, Kent) at a dilution of 1 : 50 in phosphate buffered saline. The slides were viewed on a Zeiss photomicroscope with epi-illumination (HBO 200 light source, interference filter 500 nm, 500 nm reflection, 40 × oil planopachromatic objective, 10 × eye piece, barrier filter, cut off below 500 nm). Polymorphs containing six or more fluorescent granules were taken to be positive (Fig. 1) and the number of positive cells per 1000 polymorphs was recorded as the Ig or C3 score.

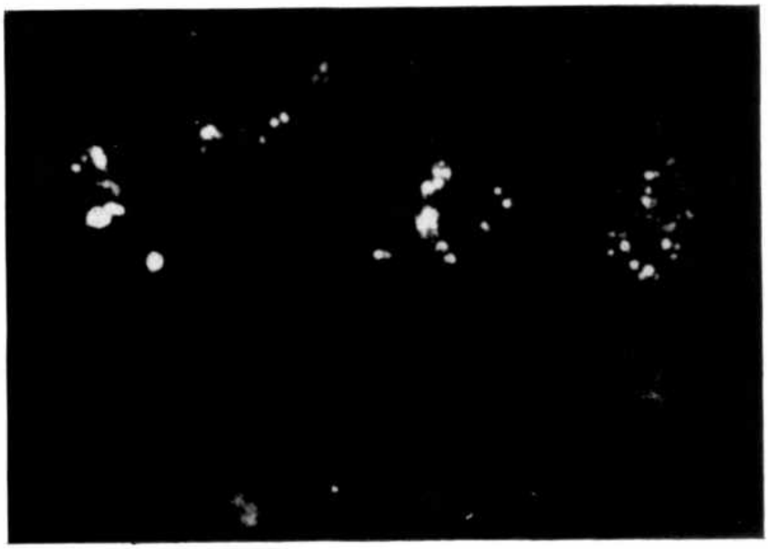

FIGURE 1. Photomicrograph of fluorescent granules within polymorphonuclear leukocytes.

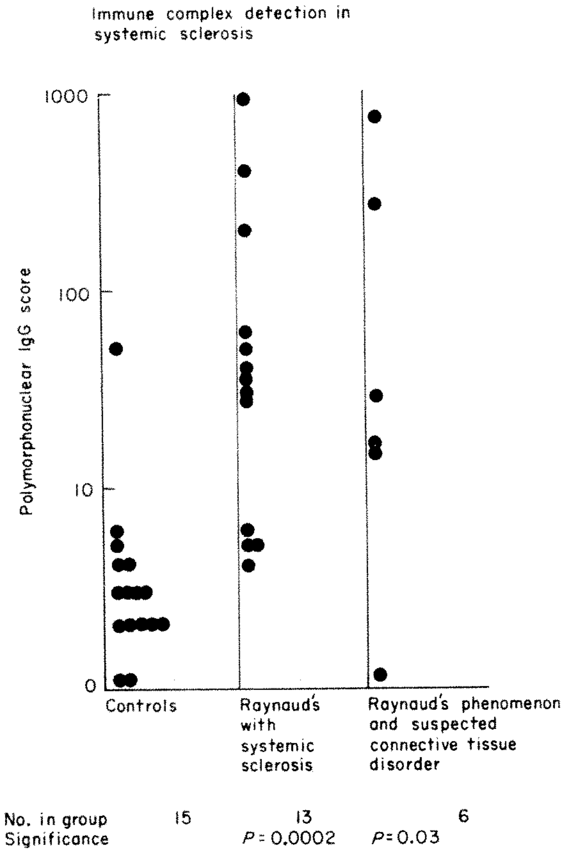

FIGURE 2. Polymorphonuclear leukocyte IgG scores.

Samples of venous blood were taken simultaneously for estimation of immune complexes by ^{125}I liquid phase C1q binding (Zubler *et al.*, 1976) using the modification of the method of Nydeggar, Lambert & Gerber, 1974, and the polyethylene glycol (PEG) precipitation method of Creighton, Lambert & Meischer (1973). Thermographic assessments of the severity of the Raynaud's phenomenon were made immediately prior to sampling in order to avoid the adverse effects of venepuncture on thermography. The severity of the Raynaud's phenomenon was assessed by means of the thermographic index (TI). This index has been developed by Cooke & Bowcock and is calculated from the formula:

$$\mathrm{TI} = t_b + (t_2 - t_0) + (t_{10} - t_b)$$

where t_b is the integrator reading at baseline, i.e. following the equilibration of the hands in an ambient temperature of 20°C, t_c the integrator number obtained immediately after stressing the hand under examination in water at 20°C for 1 min and t_2 and t_{10} the integrator numbers 2 and 10 min following cold stress. The integrator numbers are corrected for a standard temperature of 30°C and the index obtained by applying weighting factors to each element of the formula. Using this formula in over 200 cases it has been found that asymptomatic normotensive adults, age range 16–74 years, fall into a Gaussian curve of thermographic index, the index varying from 10–16 and peaking at 12, whilst patients with primary or secondary vasospastic disease of the hands have a lower index which indicates the severity of the disease.

Curves of the stressed response, plotting corrected thermographic integrator numbers against time, show the clear difference between the vascular response of the hands of normal patients and those with vasospastic disease. The normals begin at a higher temperature, the response to cold stress is dramatic, but recovery is rapid with often an 'overshoot' at or before 10 min. In contrast the range of vascular response in vasospastic disease is small and recovery is retarded sometimes even being measured in hours rather than minutes.

RESULTS

Polymorphonuclear immune complex scores

Nine of the thirteen patients with systemic sclerosis and four of the six patients with Raynaud's phenomenon suspected to be secondary to a connective tissue disease, compared with only one of the controls, showed significantly elevated levels of phagocytosed IgG, the upper limit of normal being taken as eight positive cells (Fig. 2). The scatter amongst the positive systemic sclerosic patients extended from 28 to 900 cells and from 15 to 710 cells in the group thought to have a connective tissue disease. The difference in IgG scores between the control group and each of the groups with Raynaud's phenomenon is significant, $P = 0{\cdot}0002$ and $P = 0{\cdot}03$ respectively. The C3 scores for each of the groups with Raynaud's phenomenon also differed significantly from the control group (Fig. 3); those patients with elevated levels of IgG also having elevated levels of C3, $P = 0{\cdot}0001$ and $P = 0{\cdot}05$ respectively.

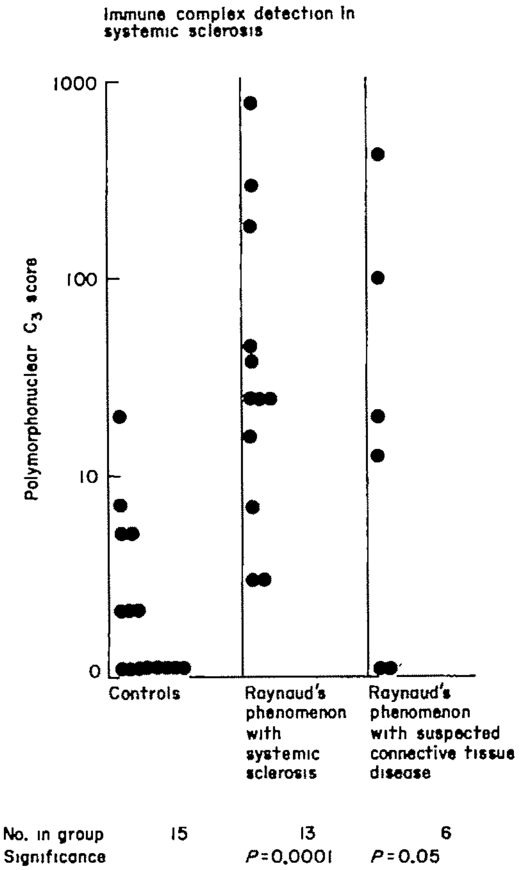

FIGURE 3. Polymorphonuclear leukocyte C_3 scores.

The correlation of IgG and C3 scores for individual patients was high, the correlation co-efficient being 0·68 for the control group and 0·95 for the group with Raynaud's phenomenon and systemic sclerosis, the *P* values being $<0·05$ and $<0·003$ respectively.

All the control patients had normal thermographic indices, the thermographic index (TI) at 10 min being within the normal range of 10–16 respectively (Table 1). Scores below the normal range represent the increasing degrees of severity of the Raynaud's phenomenon response to cold stress.

TABLE 1. Correlation of thermographic indices and IgG and C_3 scores—controls

IgG score	C_3 score	Thermographic index (2 min)	Thermographic index (10 min)
11	9	3	14
5	4	4	12
3	0	3	14
0	0	4	14
1	0	3	13
2	0	3	12
50	20	3	11
2	0	3	14
2	0	3	12
1	0	3	12
1	0	3	13
0	0	4	14
2	1	3	12
3	1	4	13
1	1	3	14

TABLE 2. Correlation of thermographic indices and IgG and C_3 scores—systemic sclerosis

IgG score	C_3 score	Thermographic index (2 min)	Thermographic index (10 min)
28	16	1	8
40	45	1	7
50	38	0	5
200	180	1	5
900	800	1	7
400	300	1	4
60	25	0	5
30	25	1	7
3	2	3	10
5	6	2	9
4	3	2	8
4	2	3	9

Within the group of patients with Raynaud's phenomenon and systemic sclerosis the vascular response to cold stress as measured by thermography at 2 and 10 min correlated inversely with the polymorphonuclear leucocyte immunoglobulin and C3 scores (Table 2). This was also the case with the group of six patients with Raynaud's phenomenon suspected to be associated with connective tissue disease (Table 3). The difference in thermographic response between each of the groups with Raynaud's phenomenon and the controls was significant both at 2 and 10 minutes after cold stress (Table 4). The Mann-Whitney-U Wilcoxon Rank sum W test showed that the inverse correlation between thermographic index and the IgG and C3 score was significant only in the group with Raynaud's phenomenon and systemic sclerosis, $P = 0{\cdot}01$ at 2 min and $P = 0{\cdot}007$ at 10 min (Table 5).

Although there was good correlation of the IgG and C3 scores in the group with Raynaud's phenomenon and suspected connective tissue disease, the correlation of the IgG score and the thermographic response was not statistically significant (Table 5). This probably reflects the small number of patients in this group. Two of the patients in this group, who showed significantly raised levels of phagocytosed immunoglobulin and complement and low thermographic indices subsequently progressed to systemic sclerosis; these are the patients whose IgG and C3 scores are marked with an asterisk (Table 3).

TABLE 3. Correlation of thermographic indices and IgG and C_3 scores: Raynaud's phenomenon suspected to be associated with a connective tissue disease

IgG score	C_3 score	Thermographic index (2 min)	Thermographic index (10 min)
16	13	3	10
15	0	3	10
710	425	0	6
275*	100	2	8
28*	20	0	5
0	0	2	10

TABLE 4. Significance of difference of the vascular response of the hands to cold challenge as measured by thermography at 2 and 10 min in patients with Raynaud's phenomenon and normal controls

	Systemic sclerosis + Raynaud's phenomenon	Raynaud's phenomenon + suspected connective tissue disease
Thermographic response at 2 min	$P = 0{\cdot}003$	$P = 0{\cdot}002$
Thermographic response at 10 min	$P = 0{\cdot}005$	$P = 0{\cdot}01$

TABLE 5. Significance of correlation of IgG and C_3 scores and of the IgG score with the thermographic response

	IgG + C_3	IgG + thermographic response at 2 min	IgG + thermographic response at 10 min
Systemic sclerosis + Raynaud's phenomenon	$P = 0{\cdot}002$	$P = 0{\cdot}01$	$P = 0{\cdot}007$
Raynaud's phenomenon + suspected connective tissue disorder	$P = < 0{\cdot}003$	0·106	0·10

Circulating immune complexes

None of the controls had raised circulating levels of immune complexes (Table 6). Two patients, one with Raynaud's phenomenon and one patient with Raynaud's phenomenon suspected to be secondary to a connective tissue disease, had circulating immune complexes detectable by raised levels of C1q binding (Table 7).

Four patients with systemic sclerosis had elevated IgG complexes detected by PEG precipitation, and one in addition had an elevated level of IgM immune complexes. A further patient with well established systemic sclerosis had only IgM complexes detected by PEG precipitation (Table 7).

In the group of six patients with suspected connective tissue disease the single patient who had elevated levels of immune complexes detectable by C1q binding also had IgG and IgM complexes detectable by PEG precipitation (Table 8). This patient went on to develop the cutaneous stigmata of systemic sclerosis. A further four patients had raised levels of IgM circulating immune complexes detectable by PEG precipitation. There was no correlation between the level of circulating and the level of phagocytosed immune complexes.

TABLE 6. Circulating immune complexes detected by C1q binding

	PEG	
C1q	IgG	IgM
9	32	20
8	22	30
7	44	50
8	46	22
6	11	14
10	22	34
11	11	16
8	26	30
9	18	22
7	40	36
9	20	18
8	34	22
8	38	26
10	50	22
9	11	14

Normal range: C1q = <15.
PEG = <60.

TABLE 7. Circulating immune complexes detected by polyethylene glycol precipitation method—Raynaud's phenomenon and systemic sclerosis

	PEG	
C1q	IgG	IgM
34	170	160
8	20	36
11	40	86
11	82	48
10	12	11
9	80	46
8	50	30
12	14	16
4	8	24
9	96	16
22	30	30

Normal range: C1q = <15.
PEG = <60.

TABLE 8. Circulating immune complexes in patients with Raynaud's phenomenon and suspected connective tissue disease detected by the polyethylene glycol precipitation method

	PEG	
C1q	IgG	IgM
9	54	110
10	—	—
12	40	110
9	37	61
12	52	80
19	80	80
14	14	60

Normal range: C1q = <15.
PEG = <60.

None of the controls or patients showed significant hypergammaglobulinaemia on routine electrophoresis. The mean IgG was 15 g/l for the controls (range 10·6–16·4 g/l) and 15.8 g/l for the patients (range 11·2–18·2 g/l), normal IgG values for our laboratory being 7·0–18·0 g/l.

The mean IgM was 1·6 g/l for the controls (range 0·6–2·6 g/l) and 1·8 g/l for the patients (range 0·8–2·4 g/l), the normal IgM values for our laboratory being 0·4–2·5 g/l.

DISCUSSION

Within the polymorphonuclear leukocytes of 69% of patients with Raynaud's phenomenon and systemic sclerosis and 66% of patients with Raynaud's phenomenon suspected at the time of presentation to be associated with a connective tissue disease we have found inclusions of IgG in good correlation with inclusions of C3. We assume therefore that these inclusions represent phagocytosed immune complexes.

The elevated levels of phagocytosed immune complexes correlated well with the severity of the Raynaud's phenomenon assessed by the thermographic index obtained by quantified infra-red thermography in a temperature controlled environment.

This is in agreement with the findings of Van der Meulen *et al.* (1979), where the elevated levels of phagocytosed immune complexes detected by the method of The *et al.* (1978) correlated with the severity of the Raynaud's phenomenon measured by photoelectric plethysmography.

Of the six patients with Raynaud's phenomenon where an underlying connective tissue disease was suspected, two of the four patients with elevated levels of phagocytosed immune complexes within one year developed the cutaneous and systemic manifestations of systemic sclerosis thus suggesting that this test may be of value in predicting the development of a connective tissue disease in those patients who present with Raynaud's phenomenon alone. Statistically systemic sclerosis is the disease which is most likely to ensue (De Takats & Fowler, 1962; Birnstingl, 1971).

Circulating immune complexes in systemic sclerosis have previously been detected by ^{125}I protein A from *Staphylococcus aureus* (Hallgren & Lind, 1976) and by a platelet aggregation test (Yanase & Imamura, 1979). By the latter method, 71% of patients with systemic sclerosis were found to have circulating IgG complexes. We have found IgG circulating immune complexes by the ^{125}I C1q binding and polyethylene glycol precipitation methods in less than 25% of the patients we investigated. However, circulating IgM-immune complexes were detected by the polyethylene glycol precipitation methods in 37% (seven of nineteen) patients with Raynaud's phenomenon, two of whom had systemic sclerosis at the time of presentation while the third subsequently developed it. Further investigations are in progress to elucidate whether phagocytosed IgM immune complexes may also be found in the peripheral blood polymorphonuclear leukocytes of these patients. None of the patients or controls had significant hypergammaglobulinaemia which might possibly have influenced the results of the immune complex assays.

Preliminary studies (performed by Dr Alun Brown) have demonstrated decreased polymorphonuclear electrophoretic mobility in patients with systemic sclerosis and Raynaud's phenomenon. This finding suggests the presence of phagocytosed immune complexes in these cells.

The finding of IgG and IgM circulating immune complexes in Raynaud's phenomenon provides a possible explanation for the reported beneficial effect of plasmapheresis in this disorder (Talpos *et al.*, 1979; Dodds *et al.*, 1979). However we found that the severity of the vasospastic response correlated well with the level of phagocytosed immune complexes, rather than with the levels of circulating immune complexes. If the beneficial effects of plasmapheresis result from clearance of immune complexes from the circulation one might have expected the reverse.

Our findings are in keeping with the hypothesis that an immunological abnormality is important in the pathogenesis of systemic sclerosis. However the nature of the stimulus to immune complex formation and the apparently paradoxical relationship of circulating and phagocytosed immune complexes in the production of the associated Raynaud's phenomenon still require explanation.

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Pancreatic exocrine function in systemic sclerosis

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SUMMARY

Lundh tests of pancreatic exocrine function were performed on twenty unselected patients with systemic sclerosis. Three patients had very low levels of tryptic activity in their intestinal juice and only nine had results which were unequivocally normal.

Eight patients had biochemical steatorrhoea, but in six this was associated with intestinal bacterial overgrowth and a seventh had primary biliary cirrhosis. The remaining patient had no cause for steatorrhoea other than the marked pancreatic insufficiency which had been demonstrated.

Although pancreatic damage may contribute to malabsorption in systemic sclerosis, it appears to be less important than other factors such as intestinal bacterial overgrowth.

Weight loss and malabsorption are recognized complications of systemic sclerosis (Stachow, 1975). Pancreatic fibrosis has been described as a pathological finding in systemic sclerosis (Piper & Helwig, 1955) and pancreatic exocrine insufficiency is a potential cause of malabsorption in systemic sclerosis. Previous studies of pancreatic function (Scudamore *et al.*, 1968; Dreiling & Soto, 1976) have tended to support this, but results have been equivocal and the patients studied were highly selected, most having gastrointestinal symptoms and radiological evidence of intestinal involvement.

There is little information about the incidence of pancreatic dysfunction in unselected patients with systemic sclerosis. We have investigated pancreatic exocrine function by measuring the trypsin output in response to a standard (Lundh) liquid meal in a group of twenty unselected patients with systemic sclerosis.

METHODS

All patients were attending the Dermatology Department of the General Infirmary at Leeds with a confirmed diagnosis of systemic sclerosis. There were eighteen women and two men with ages ranging from 15 to 67 years. All had Raynaud's phenomenon and typical acrosclerotic skin changes. The degree of visceral involvement, and hence general disease severity, was quantitatively assessed in each patient using the criteria described by Hughes *et al.* (1978). This index makes use of a scoring system with points allotted on the basis of involvement of skin, lungs, gastrointestinal tract (in

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particular radiological involvement of oesophagus and/or colon), heart, kidneys, or the presence of other abnormalities, e.g. Sjögren's syndrome. The higher the 'score' for a given patient, the greater degree of systemic involvement. Patients were questioned about gastrointestinal symptoms, in particular weight loss and diarrhoea, and the routine biochemical investigations carried out included levels of fat soluble vitamins (or related indices such as the prothrombin time) and faecal fats (PEG-4,000 marker method). All patients had had small bowel barium meals as described by Lintott (1978), a breath test for intestinal bacterial overgrowth (Metz *et al.*, 1976; Cobden *et al.*, 1980), and seventeen had had a successful small bowel biopsy.

Lundh test meals were carried out in the fasting state as previously described (Lundh, 1962; Ashton, Axon & Lintott, 1978). A sample of intestinal juice was aspirated and sent for aerobic and anaerobic culture (Cobden *et al.*, 1980) before the test meal was given. The mean tryptic activity (MTA) of the juice collected in the subsequent 2 hours was measured by standard methods (Ashton *et al.*, 1978; Cook *et al.*, 1967), the result being expressed as International Units of tryptic activity per ml of juice (iu/ml). Duplicate assays were performed and the mean of the two results taken as the MTA. In practice, the two assays rarely differed by more than 10%, the greatest discrepancies occurring in those samples with the highest MTA values (i.e. those which were most clearly normal) when such differences are of no clinical relevance.

RESULTS

Mean tryptic activities ranged from 5·0 to 28·0 iu/ml. Three values were in the range associated with gross pancreatic disease and only nine were in the unequivocally normal range (see Fig. 1).

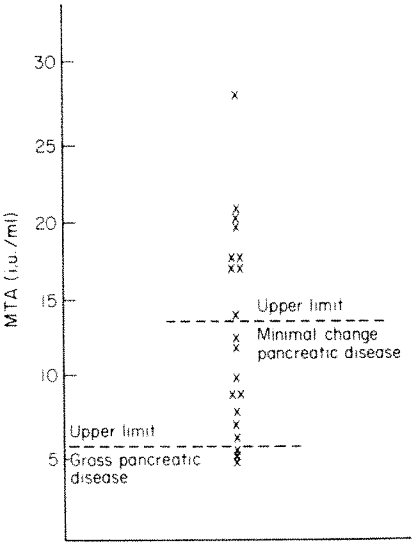

FIGURE 1. Mean tryptic activities (iu/ml) of intestinal juice in twenty patients with systemic sclerosis. - - - indicates upper limits of ranges for gross pancreatic disease and minimal-change pancreatic disease (see text).

Table 1 shows the results for biochemical and nutritional values for each patient. The MTA values and symptomatology are also shown, and there are columns indicating whether or not the patient had abnormalities on radiology, jejunal histology or evidence of intestinal bacterial overgrowth. The results for the serum calcium and prothrombin times are not shown, being normal in all patients.

TABLE I. Clinical and biochemical details of patients studied

Patient	Age	Symptoms		Visceral involvement index (scoring—see text)	Lundh MTA (iu/ml)	Faecal fats (NR < 18 mmol/l)	Intestinal bacterial overgrowth	Jejunal biopsy	Small bowel radiology	Vit A (NR > 1·1 micro-mol/l)	Alk Phos (NR > 13 ka units)	Mg (NR 0·7–1·0 mmol/)	Zn (NR > 12·6 micro-mol/l)
		Wt. loss	Diarrhoea										
1 jm ♀	41	—	—	6	20·3	3·0	—	n	n	4·1	5·1	0·77	8·2
2 kj ♀	62	+	—	17	14·1	27·3	—	a	n	2·3	41·0*	0·95	7·8
3 jf ♀	19	—	—	3	5·0	27·0	—	n	a**	4·5	10·0	0·80	10·6
4 bb ♀	35	—	—	9	28·0	12·3	—	n	n	(—)	61·0*	(—)	12·8
5 jt ♀	40	—	—	2	20·0	17·0	—	n	n	2·6	9·0	0·80	(—)
6 jb ♀	55	+	—	9	10·1	16·0	—	a	n	4·0	8·3	0·53	11·2
7 kp ♀	58	—	—	12	17·1	3·1	—	n	n	4·2	8·6	0·78	(—)
8 dp ♀	56	—	—	9	9·2	11·0	—	a	n	2·5	7·9	8·89	(—)
9 pc ♀	46	+	+ +	12	17·1	210·0	+	n	n	3·9	4·9	0·86	(—)
10 lm ♀	62	+	+	15	20·9	8·8	—	n	n	2·0	7·8	0·71	9·0
11 ma ♀	59	—	+ +	12	5·6	(—)	—	n	n	3·9	8·5	0·83	9·2
12 hs ♀	63	+ +	—	9	17·8	30·0	+	a	a	0·9	6·0	0·77	7·4
13 km ♀	15	—	—	5	12·8	10·1	—	n	n	1·9	13·7	0·80	13·6
14 fp ♀	67	+	+ +	8	8·3	20·0	+	n	n	2·3	7·4	0.87	9·8
15 fg ♂	58	—	—	12	6·5	(—)	—	n	n	(—)	12·2	(—)	(—)
16 ba ♀	55	+	—	2	9·2	9·0	+	n	n	2·0	8·8	0·88	8·4
17 ca ♀	31	—	+	9	7·3	18·9	+	n	n	3·5	6·7	0·80	17·0
18 mb ♀	61	—	—	8	12·2	34·0	+	(—)	a	2·7	12·8	0·99	18·0
19 jd ♀	20	+	+	12	5·3	14·1	—	(—)	n	(—)	6·3	(—)	(—)
20 mc ♀	51	—	+ +	15	17.8	22·0	+	(—)	a	3·0	10·7	0·89	8·4

n = normal; a = abnormal; (—) = not tested; + = present; — = absent; NR = normal range; * = primary biliary cirrhosis; ** = prolonged transit only. Figures underlined are abnormal

DISCUSSION

The Lundh test is well established as a simple means of assessing exocrine pancreatic function with a good diagnostic success rate in detecting severe pancreatic disease (Lundh, 1962; Ashton *et al.*, 1978; James, 1973). Focal pancreatic disease, or lesser degrees of damage, have been recognized for some time and recently Ashton *et al.* (1978) have been able to demonstrate a correlation between structural changes in the pancreas, seen at ERCP, and the MTA values obtained by the Lundh test. They defined three groups of patients on the basis of the pancreatogram findings. One group comprised patients with normal pancreatograms, a second consisted of patients with gross pancreatic disease and the third group of patients had pancreatograms showing focal, or mild diffuse, abnormalities which they classified as 'minimal-change pancreatic disease'. Patients with gross disease had consistently low values and the authors were able to define two limiting values for the test. MTA values below 6·0 iu/ml were felt to be indicative of gross pancreatic disease, while values above 14·0 iu/ml were found only in patients with unequivocally normal pancreatograms. Between these values, results could be associated with either a normal pancreas or minimal-change disease.

Applying these criteria to the results obtained in this study from the same Unit, there were nine patients with unequivocally normal MTA values. Three patients had values indicative of gross pancreatic disease. Somewhat surprisingly, there appeared to be no relationship between the general severity of the systemic sclerosis in each patient, as assessed by the degree of systemic involvement, and pancreatic exocrine function. Thus, one patient (No. 3) with a very low MTA value had little evidence of visceral involvement, and equally there were patients (e.g. Nos. 2, 10 and 20) with widespread systemic disease and normal pancreatic function.

From the clinical and biochemical details of the patients (Table 1) it can be seen that eight patients had proven steatorrhoea, but in six this could be accounted for by a 'blind-loop' sydrome due to intestinal bacterial overgrowth (Cobden *et al.*, 1980), and another patient (No. 2) had primary biliary cirrhosis. The remaining patient (No. 3) had no evidence of overgrowth and had normal small bowel histology, but had a a very low MTA value of 5·0 iu/ml. In the absence of any other known cause, it seems very likely that her steatorrhoea was related to pancreatic exocrine insufficiency. Of the two other patients with low MTA values, one (No. 19) did not have steatorrhoea. The other (No. 11) had a history of diarrhoea which might well have indicated steatorrhoea, but unfortunately a successful faecal fat collection was not achieved.

There was little evidence of impairment of absorption of fat related nutrients. All patients had a normal serum calcium and prothrombin time. Three had elevated alkaline phosphatase levels but two of these had primary biliary cirrhosis and the other was an adolescent girl in whom the elevation was presumably physiological and related to pubertal growth changes. One patient with intestinal overgrowth had hypomagnesaemia, and another with an abnormal jejunal biopsy had a low vitamin A level. Ten of the fourteen plasma zinc levels measured were low, but the significance of this is uncertain.

Dreiling & Soto (1976) reported abnormal pancreatic secreting patterns in 61% of their systemic sclerosis patients, including two out of the five without gastrointestinal symptoms. In our unselected group, Lundh test evidence of severe pancreatic exocrine insufficiency was found in only three (15%) of twenty patients. It is possible that others may have mild or focal pancreatic damage, but the potential for improvement in nutritional status with enzyme replacement therapy would appear to be limited. Whilst confirming that pancreatic disease is a cause of malabsorption in systemic sclerosis, it is clearly not as common or important a factor as intestinal bacterial overgrowth (Cobden *et al.*, 1980).

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Zoon's balanitis (balanitis circumscripta plasmacellularis)

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SUMMARY

The clinical and histopathological features of nineteen cases of Zoon's balanitis and a comparable lesion of the vulva have been reviewed. The condition is particularly distinguished by characteristic histological changes affecting the epidermis and the dermal vasculature. The presence of a plasma cell infiltrate is relatively non-specific. Recognition of these features should allow simple differentation from premalignant, infective and other inflammatory penile lesions which may be more responsive to treatment.

Since the original description of a distinctive balanitis by Zoon (1952), there have been only sporadic case reports including, most recently, those by Brodin (1980) and Stern & Rosen (1980). Nevertheless, the typical clinical features are readily recognized by dermatologists and the entity is further distinguished by a characteristic histology. The differentiation of circumscribed penile lesions is important to exclude incipient malignancy and to identify lesions which are amenable to specific treatment.

The case reports and histological material from thirty patients seen over a 20-year period were reviewed. In each instance (excepting one female patient) the original clinical and pathological diagnosis had been plasma cell balanitis (Zoon). Histological sections stained with haematoxylin and eosin (H & E), Fontana silver stain for melanin, Prussian blue (B.P.), Weigert's stain for elastic tissue, periodic acid Schiff reagent (PAS) and for chloracetate esterase activity, (Leder, 1964) were examined. Nineteen of the thirty cases proved to have Zoon's balanitis and one further case was an equivalent lesion of the female vulva. The remaining ten patients showed a range of histological features and included cases of herpes simplex, Candidiasis, lichen planus, erythroplasia of Queyrat and non-specific balanitis.

CLINICAL FEATURES

In the male patients the lesion was present on the glans penis in seventeen cases and on the mucosal

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aspect of the prepuce and in the coronal sulcus respectively in the other two. The lesions were solitary asymptomatic circumscribed macules with a maximum diameter of 3 cm. Characteristically a shiny glazed orange-coloured macular erythema with multiple pinpoint brighter red spots ('Cayenne pepper spots') was present (Fig. 1), although in some instances only a vague clinical description was documented. The duration of history ranged from 4 months to 12 years. All of the patients were uncircumcised and varied in age from 34 to 75 with a mean of 56 years. In every case topical steroid and/or antifungal applications had been used and found to be largely ineffective, although some success with potent topical steroids was reported in two cases.

The single female patient, aged 50, presented with a pruritic localized erythematous erosion on the inner aspect of the right labium minus.

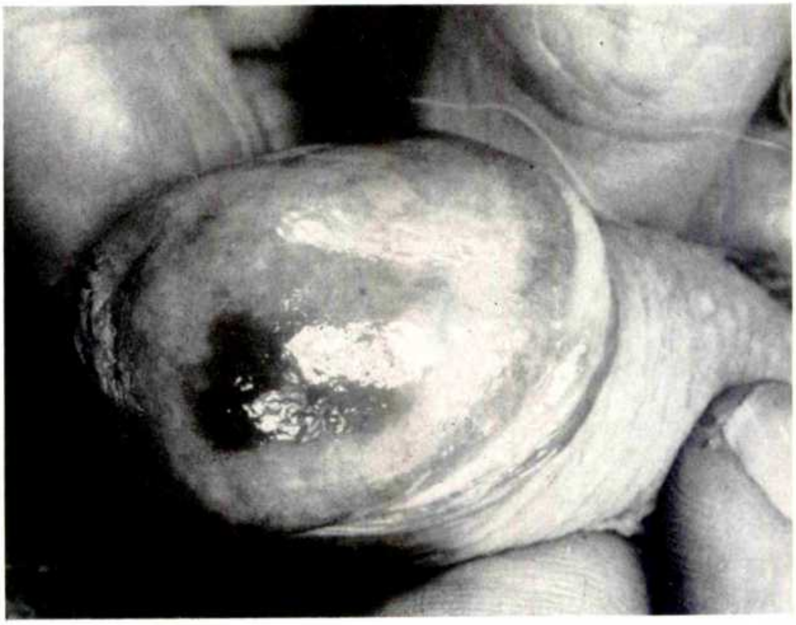

FIGURE 1. Shiny, glazed macular erythema on the glans penis.

HISTOPATHOLOGY

The histological features of our cases of Zoon's balanitis were distinctive and uniform. The epidermis was usually attenuated and in a minority of cases frankly ulcerated, although epidermal hyperplasia was apparent in a single case and occasionally present at the margin of lesions. There was complete effacement of the rete ridge pattern (Figs 2 and 3). Epidermal thinning was due to reduction in size and numbers of keratinocytes and absence of the horny and granular cell layers. The architecture of the epidermal cells was striking and provided the single most characteristic feature of the histology. Individual keratinocytes, particularly in the suprabasal layers, were uniformly diamond-shaped with their horizontal axis exceeding the vertical, an appearance which we termed 'lozenge keratinocytes', (Figs 2 and 3). Occasional dyskeratotic cells were present but atypia and mitoses were absent. In the affected areas, throughout the epidermis, there was regular widening of the intercellular spaces between individual keratinocytes presumably due to oedema, further adding to the characteristic

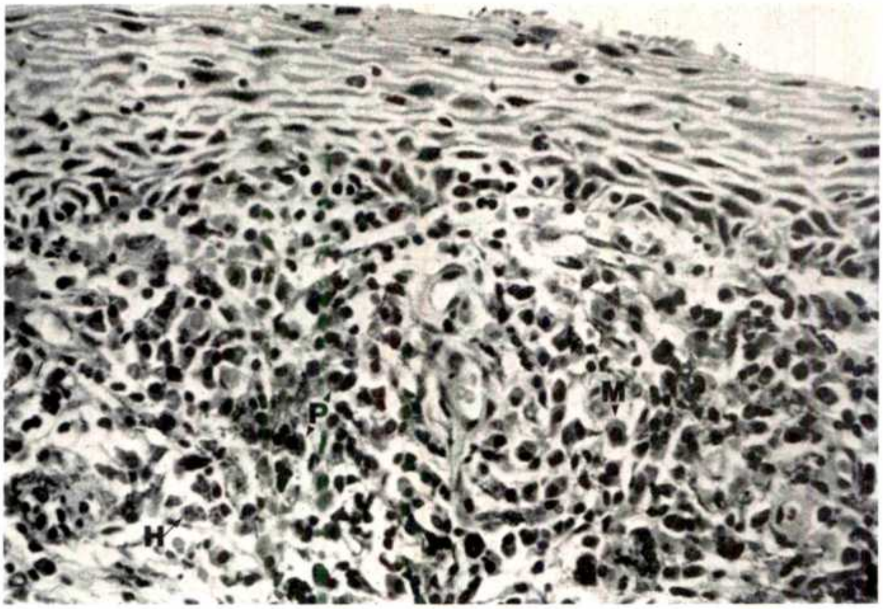

FIGURE 2. Zoon's balanitis (H & E, × 280). Epidermis comprises 'lozenge keratinocytes' separated by uniform intercellular oedema. There is loss of the granular cell layer and effacement of the rete ridge pattern. The upper dermal infiltrate includes a preponderance of plasma cells (P), haemosiderin laden macrophages (H) and mast cells (M).

appearances: we termed this feature 'watery spongiosis' (Figs 2 and 3). Frank vesiculation was never present. In certain areas, erythrocytes or polymorphonuclear leucocytes were sparsely scattered within the epidermis (Fig 3, inset), and occasional exocytosis of mononuclear cells particularly in the basal and suprabasal layers of the epidermis was seen (Fig 2).

In the dermis, a dense band-like or lichenoid infiltrate was present, frequently extending to the mid-reticular dermis. Plasma cells were predominant in the infiltrate and usually exceeded 50% of the cells present (Figs 2 and 3), although the plasma cell population was assessed as low as 15% and 20% respectively in two cases. An appreciable number of lymphocytes were constantly present, sometimes in quite dense focal collections (Fig. 3, inset). Mast cells were readily visible in most cases, sometimes comprising up to 5% of the cell population (Fig. 2). This was readily appreciated on chloracetate esterase staining. Occasional eosinophil and polymorphonuclear leucocytes were also seen.

Changes in the dermal vasculature were prominent and characteristic. There was considerable vascular proliferation with singular vertical or oblique orientation of dilated individual vessels (Fig. 3). Haemosiderin deposition was frequently marked (Figs 2 and 3), but occasionally could not be found even in B.P. stained specimens. Free extravasated erythrocytes were often abundant in the dermal infiltrate.

In some cases the dermal infiltrate was less marked and in these instances a degree of fibrosis and loss of elastica with easily visible fibroblasts could be seen.

PAS staining highlighted the presence of polymorphonuclear leucocytes in the epidermis but no mycotic organisms were seen. Staining for melanin was unremarkable.

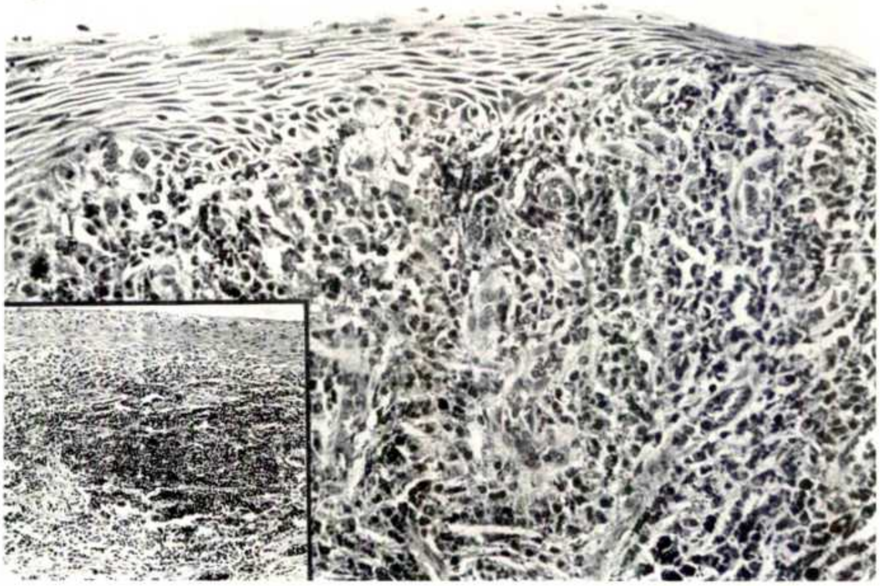

FIGURE 3. Zoon's balanitis (H & E, × 175). The epidermis shows absence of granular layer, loss of rete ridge pattern, 'lozenge keratinocytes' and watery 'spongiosis'. Prominent vertical and oblique blood vessels and heavy haemosiderin deposition are present in the upper dermis. The cellular infiltrate comprises principally plasma cells. (Inset) A focal collection of lymphocytes is present in the upper dermis. Epidermal infiltration by neutrophils can be seen (H & E, × 42).

DISCUSSION

Zoon (1952) reported eight cases of a benign inflammatory balanitis which he differentiated from erythroplasia of Queyrat. Many of the characteristic histological features, including epidermal atrophy, spongiosis, loss of rete ridge pattern, lichenoid dermal infiltrate and vascular proliferation, were detailed in this original description. The plasmacytic nature of the infiltrate was particularly stressed giving rise to the terminology, plasma cell balanitis, which is in common use. Plasma cell numbers in the dermal infiltrate of our cases were widely variable, however, suggesting that this is a relatively non-specific feature. The inflammatory response to mucosal lesions frequently contains a preponderance of plasma cells, which therefore may be expected to be numerous in any penile lesion (Korting & Theisen, 1963).

The histological features of Zoon's balanitis are distinctive. The characteristic epidermal changes of atrophy, loss of rete ridges, 'lozenge keratinocytes' and 'watery spongiosis' are perhaps unique to this condition. The appearances are further distinguished by the subepidermal location of the inflammatory infiltrate, the proliferation and vertical orientation of dermal vasculature, erythrocyte extravasation and haemosiderin deposition. Despite this typical histopathology, 30% of the cases reviewed, which were originally considered to be Zoon's balanitis, had been incorrectly diagnosed histologically.

Clinical differential diagnosis includes several conditions which are amenable to treatment. Lichen planus, psoriasis, Reiter's disease, fixed drug eruption, herpes simplex, secondary syphilis and Candidiasis may all pose diagnostic difficulties. Early lesions may be impossible to differentiate from erythroplasia of Queyrat. Nevertheless, the typical established case is usually obvious; chronicity and poor response to topical treatment may provide further clues. Histologically, lichen planus, secondary syphilis and candidiasis may show features in common with Zoon's balanitis but lack the typical changes in the epidermis and dermal vessels. It is particularly important, but relatively simple histologically, to differentiate the dysplastic epithelium of erythroplasia of Queyrat from the epidermal changes of Zoon's balanitis. Cases of so-called lichenoid, telangiectatic and purpuric balanitis and vulvitis (Jonquieres & de Lutzky, 1980) share many similar histopathological features with Zoon's balanitis and probably represent cases of the latter which lack some of the most characteristic changes.

The aetiology of the condition is poorly understood. In our cases the lesion occurred exclusively in the uncircumcised. Chronic infection by *Mycobacterium smegmatis* has been postulated (Montgomery, 1967), and heat, constant friction and poor hygiene may be predisposing factors. Circumcision is frequently curative.

The occurrence of an equivalent lesion of the female genitalia has been previously described (Garnier, 1957; Hyman & Leider, 1961; Grimmer, 1967), and comparable lesions may also occur around other mucosal orifices (Korting & Theisen, 1963). The plasma cellular infiltrate is probably non-specific, but the term plasmacytosis circumorificalis remains appropriate in these cases until specific aetiological factors are determined.

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Pharmacology and Treatment

The influence of 'Tigason' (Ro 10-9359) on the serum lipoproteins in man

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SUMMARY

Eight patients with psoriasis or Darier's disease were studied during 1–3 months of 'Tigason' treatment (0·7–0·8 mg/kg body weight). The levels of triglycerides and cholesterol in whole serum and in different lipoprotein fractions were repeatedly determined. Before treatment all lipid values were within the normal range. During treatment the values changed gradually and five of the eight patients developed pathological VLDL triglyceride concentrations. A significant increase of VLDL cholesterol and a decrease in the HDL cholesterol was also noted. Until more information is available, prolonged treatment periods with 'Tigason' should perhaps be avoided, at least in high risk patients.

An aromatic retinoid ('Tigason', etretinate) has been found to be a valuable drug in the treatment of psoriasis, Darier's disease and other disorders characterized by disturbed keratinization. The toxicity of retinoids in high doses is well known but also with lower doses there are side effects which must be taken into consideration.

During treatment with 'Tigason' an increase of the serum triglyceride concentration has been observed in most of our patients. The present study was undertaken to analyse in more detail the influence of this drug on the serum lipoprotein composition.

MATERIALS AND METHODS

Patients

Eight patients (seven men and one woman) with psoriasis or Darier's disease participated. They were otherwise healthy, used no other drug and had no known disturbances of lipid metabolism. A daily dose of 'Tigason' (0·7–0·8 mg/kg body weight) was given for 1–3 months. During this time there was no significant change of the body weight and the patients maintained their normal food and alcohol intake.

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Lipoprotein analyses

The lipoprotein lipid concentrations were determined in serum after a 12–14-h overnight fast. EDTA was added to serum as a 5% solution to give a final concentration of 0·05%. The very low density lipoprotein lipids (VLDL) were isolated as the top fraction after centrifugation at d = 1·006 for 16 h at 15°C and 105,000 *g* in a Beckman LS-65B preparative ultracentrifuge using a 40·3 rotor (Havel, Eder & Bragdon, 1975). The low density lipoproteins (LDL) were precipitated from the bottom fraction at d = 1·006 by a heparin-manganese chloride solution. After low speed centrifugation, the high density lipoprotein (HDL) lipid levels were determined in the supernatant (Burstein & Samaille, 1960). The concentrations of the lipids in LDL were obtained indirectly by subtracting the HDL lipid levels from lipid concentrations of the bottom fraction after centrifugation at d = 1·006.

Cholesterol and triglyceride concentrations in serum and in the isolated lipoprotein classes were determined in isopropanol extracts by semi-automated methods in a Technicon AutoAnalyzer type II (Rush, Leon & Turrell, 1971). The recoveries of triglyceride and cholesterol in the isolated density classes were within 100 ± 10% of the serum values in all samples. The top and bottom fractions at d = 1·006, as well as whole serum were analysed using agarose gel electrophoresis (Noble, 1969). This was done in order to check the purity of the isolated lipoprotein fractions as well as to exclude the presence of certain dyslipoproteinaemias, e.g. hyperlipoproteinaemia type III and certain other abnormal lipoprotein fractions.

The concentrations of serum apolipoproteins (apo) B, A-I and A-II were determined by electroimmunoassay according to Laurell (1972) modified as suggested by Curry, Aloupovic & Svenram (1976). The details of the assay procedure have been described earlier (Vessby *et al.*, 1980). The apolipoprotein concentrations in serum are expressed in arbitraty units (a.u.) relative to the concentration in the reference serum obtained from a large pool of healthy blood donors (100 a.u.).

Statistics

The data presented are based on individual comparisons between results recorded before and after treatment with 'Tigason'. The significance of the differences between mean values was estimated with a paired t-test (two-tailed test).

RESULTS

The mean values for lipoprotein lipid and serum apolipoprotein concentrations before and after treatment are listed in Table 1. All patients, regardless of their initial triglyceride values, showed increasing VLDL triglycerides during treatment. The percentage increase seemed to be approximately the same in those with initially low values as in those with higher pretreatment values. This tendency to increased VLDL triglyceride concentrations was already seen at 2 weeks in the two patients checked at that time. In four patients investigated both after 1 month and after 2–3 months of treatment on the same dosage, there was a continued increase of the triglyceride value. Before treatment two of the eight patients had VLDL triglycerides at the upper normal range. After treatment five of the patients had VLDL triglyceride concentrations above the cut-off point for normality. There was also a marked increase of the VLDL cholesterol during treatment. Concomitant with the increasing VLDL levels there was a significant decrease in the HDL cholesterol concentration by, on average, 12%.

The mean serum concentrations of apo B, A-I and A-II did not change during treatment with Tigason. Apo B is the major protein constituent of LDL and only a minor proportion of the serum

TABLE 1. Lipoprotein composition (mean ± s.d.) before and after treatment with 'Tigason' (50–60 mg/day, n = 8) for 1–3 months

	VLDL		LDL			HDL				Whole serum	
	TG	CH	TG	CH	Apo B	TG	CH	Apo A-I	Apo A-II	TG	CH
Before treatment	0·75 ± 0·38	0·39 ± 0·24	0·45 ± 0·21	4·00 ± 0·87	130 ± 30	0·21 ± 0·07	0·25 ± 0·35	98 ± 17	100 ± 14	1·60 ± 0·91	5.66 ± 1·01
After treatment	1·92 ± 1·08	0·95 ± 0·49	0·48 ± 0·18	3·98 ± 0·90	136 ± 31	0·23 ± 0·06	1·10 ± 0·34	99 ± 14	100 ± 11	2.71 ± 1·25	5·97 ± 1·17
% Increase	+152	+162	+14	+0		+11	−12			+77	+6
P	< 0·001	< 0·002	N.S.	N.S.	N.S.	N.S.	< 0.02	N.S.	N.S.	< 0·001	N.S.

TG = Triglyceride concentration mmol/l; CH = cholesterol concentration mmol/l; Apo B, A-I and A-II = apolipoprotein B, AI and A-II concentration, respectively in arbitrary units; *P* = statistical significance, paired t-test (two-tailed test).

N.S. = Not significant.

apo B is present in VLDL (Albers, Cabana & Hazzard, 1975). Thus the unchanged levels of apo B are in accordance with the unchanged concentration of LDL during treatment. Apo A-I and A-II are nearly exclusively confined to HDL (Curry *et al.*, 1976). Thus the unchanged apo A levels, in combination with the significantly reduced HDL cholesterol concentration, indicates that a change of the HDL composition with a relative cholesterol depletion may have occurred during treatment with Tigason.

The same tendencies have also been observed in patients on a daily dosage of 25 mg, although less pronounced than in those on 50–60 mg daily. It is presently not known if the changes of the lipoprotein pattern recorded during 'Tigason' treatment are apparent also during long-term treatment We have had only one patient on retinoid alone for more than 6 months. This patient, however, had a progressive increase of his triglyceride level from 1·4–3·9 mmol/l in whole serum. The VLDL triglyceride value was 2·93 mmol/l after 6 months. The time required for normalization of the lipid values seems to vary, but a post-treatment change towards normal has been seen in several patients within 2–4 weeks.

DISCUSSION

A report on hypertriglyceridaemia in rats given all-trans- as well as 13-cis-retinoic acid has recently been published by Gerber & Erdman (1980). They found no significant changes in liver phospholipids, cholesterol or glyceride concentrations but a pronounced increase of the triglycerides in serum. Observations by Peck (1989) and Nigra (1980) indicate that 13-cis-retinoic acid ('Isotretinoin') can induce similar changes in human serum and that the increase of the triglycerides is most marked in the VLDL fraction. Recently eruptive xanthomas were reported in a patient on treatment with 'Isotretinoin' (Dicken & Connolly, 1980).

Although 'Isotretinoin' and 'Tigason' differ in some of their biological effects, both of the drugs obviously influence the serum lipid composition. Increased serum triglyceride concentrations (Carlson & Böttiger, 1972) as well as decreased HDL cholesterol levels (Gordon *et al.*, 1977; Miller *et al.*, 1977) have been reported to be risk factors for development of coronary heart disease. When considering the optimal dosage and duration of treatment with 'Tigason' it seems important to bear these effects in mind. Until more information is available prolonged treatment periods should perhaps be avoided, at least in high risk patients.

Further studies are now under way on the mechanisms of the influence of the retinoids on the lipids and on possible ways to modify this influence.

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Case Reports

Unilateral systematized keratosis follicularis. A variant of Darier's disease or an epidermal naevus (acantholytic dyskeratotic epidermal naevus)?

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SUMMARY

In this report seven new cases showing unilateral systematized, linear or zosteriform distribution of lesions resembling Darier's disease, but without other findings suggestive of Darier's disease, are presented.

It is suggested that the eruption represents an epidermal naevus for which the name acantholytic dyskeratotic epidermal naevus is proposed.

Classical keratosis follicularis (Darier's disease) is a well established clinical entity with characteristic histological features of acantholysis and dyskeratosis. However, as pointed out by Ackerman (1972) these features are not pathognomonic for Darier's disease and may also be present in other entities.

In the literature a number of patients have been reported presenting with crusted keratotic lesions in unilateral, systematized, linear or zosteriform distribution and with the histological features traditionally associated with Darier's disease. Classification of these cases as a variant (forme fruste) of Darier's disease or as an epidermal naevus is still a matter of dispute.

In this study seven new cases will be presented.

CASE REPORTS

Case 1

A 47-year-old man has had an asymptomatic band-like papular eruption on the left hemithorax and upper arm, which started on the flank 8 years before and which is still extending. Sunlight does not aggravate the condition.

On examination several hundreds of 1–4 mm crusted follicular papules are present. The eruption runs as a single 1–2 cm wide band from the posterior aspect of the left upper arm across the shoulder obliquely down to the midline, from where it fans out in four waving, S-shaped bands towards the left lower lateral and anterior thorax (Fig. 1).

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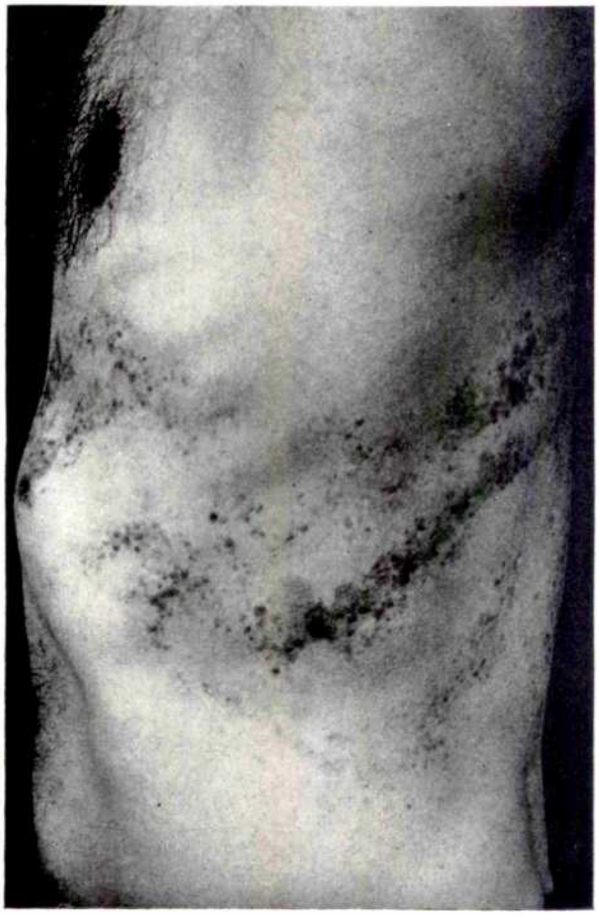

FIGURE 1. Crusted keratotic papules following the lines of Blaschko on the left hemithorax in case 1.

Case 2

A 27-year-old man. For 4 years he has had a papular eruption on the left shoulder and left lower chest that started in summer.

On examination about 100 1–2 mm follicular pink papules with fine, comedo-like keratoses are present on the left scapular area extending to the supraclavicular fossa. A second zosteriform area about 5 cm wide with similar papules is present on the left lower chest extending from the spine to the xiphoid.

Case 3

A 58-year-old man. For 5 years he has had a slowly extending papular eruption on the right hemithorax and upper abdomen following trauma. Hot weather and perspiration aggravate the condition and cause severe itching while exposure to sunlight alone does not.

On examination several hundred pinpoint follicular, keratotic papules are present on the right of the chest and upper abdomen. The lower anterior chest just right of the midline shows an almost square palm-sized area extending in an ill-defined 2 cm wide band towards the anterior axillary fold. A branching S-shaped band-like lesion similar to that of case 1 is present on the lateral aspect of the right lower chest. Furthermore he has an inverted U-shaped area to the right of the umbilicus and two isolated nummular patches on the right upper and lower scapular angles.

Case 4

A 46-year-old red-haired man. For 22 years he has had a papular eruption on the lower back that started during a stay in West Iran. A second papular area has been present on the upper back for 14 years following exposure to sunlight. Both areas slowly extended for some years. Perspiration aggravates the condition and causes some itching, whilst exposure to sunlight does not.

Examination reveals a palm-sized area with reticular hyperpigmentation and grouped and confluent brownish-red keratotic papules on the lower back just to the right of the midline. A second crescent-shaped, palm-sized area with similar papules is present on the right upper back.

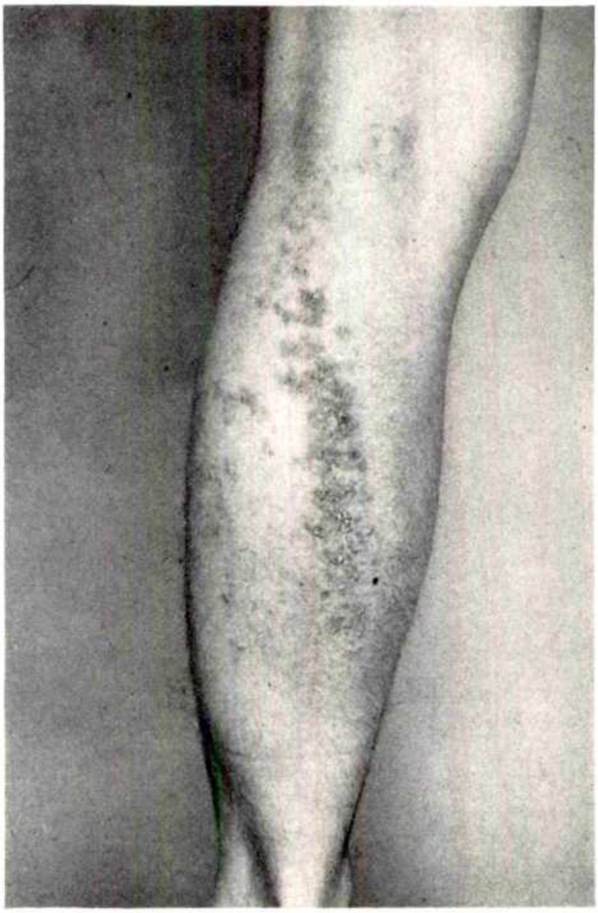

FIGURE 2. Linear keratotic lesions on the left lower leg in case 5.

Case 5

A 17-year-old woman. For 9 months she has had a non-itchy, slowly extending papular eruption on the left leg that started on the calf. Sunlight does not aggravate the condition.

On examination there is a linear eruption on the posterior aspect of the left lower leg extending from the popliteal fossa to mid-calf, with several hundreds of erythematous lichenoid and crusted papules. The central area on the calf shows a warty appearance (Fig. 2). On the lateral aspect of the left lower leg, the dorsum of the left foot and the dorso-lateral aspect of the left upper leg several papular patches are present.

Case 6

A 77-year-old man. For 15 years he has had an itchy eruption, almost identical to case 5, on the left leg.

Case 7

A 47-year-old man presented with an irregular, oblong, palm-sized, red and papular area with fine psoriasiform scaling and a number of excoriations on the proximal part of the lateral aspect of the left lower arm.

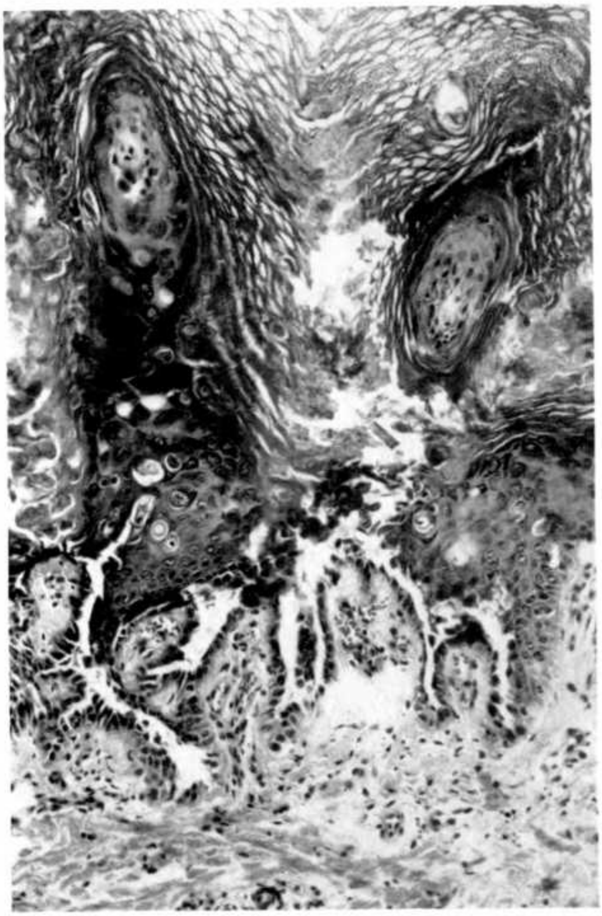

FIGURE 3. Biopsy from the warty area in case 5 showing the typical features of focal acantholytic dyskeratosis.

The eruption started about 20 years earlier. After exposure to sunlight and during hot moist weather the area becomes itchy and sometimes eczematous.

There was no other abnormality of the skin or oral mucosa in any of the patients. All patients denied having a family history of a similar condition.

HISTOLOGICAL EXAMINATION

Biopsies were taken from the keratotic lesions in all seven patients. All revealed acanthosis, papillomatosis, hyperkeratosis, focal parakeratosis, hypergranulosis, and suprabasal acantholysis with, in some biopsies, formation of villi and lacunae, as well as grains and corps ronds. Epidermal changes were most prominent in the biopsy from the verrucous areas in cases 5 and 6. The upper dermis showed dilated blood vessels, and a slight, mainly lymphocytic, infiltration (Fig.3).

ELECTRONMICROSCOPY

A biopsy was taken from the chest in case 1. The tissue was dehydrated and embedded in a mixture of Epon and Araldite, after fixation in 2% cacodylate-buffered glutaraldehyde and postfixation in 1% osmium tetroxide. The sections were counterstained with uranyl acetate followed by lead citrate and examined on a Siemens Elmiscope 51.

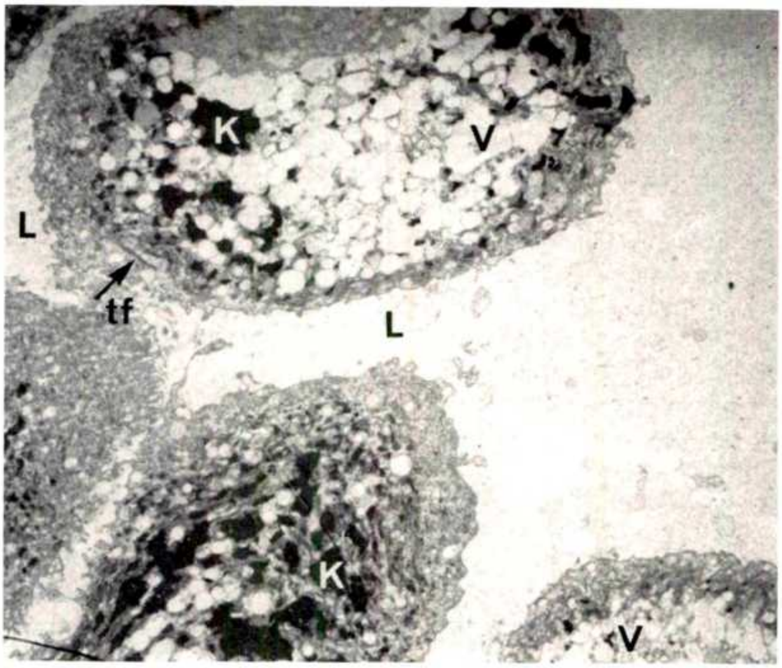

FIGURE 4. Electron photomicrograph showing lacunae (L) and round cells with diffusely vacuolated cytoplasm (V), so-called 'corps ronds'. The nuclei are no more intact. The tonofilaments (tf) appear compressed at the cell periphery. There are many keratohyalin granules (K) of varying size (× 7500).

The dermis and basement membrane are normal. In the basal layer the intracellular spaces appear to be wider than normal with partial loss of cellular attachment. Dyskeratosis and acantholysis start in the spinous layer with retraction of the tonofilaments from the cell surface and aggregation around the nucleus. In the granular layer most desmosomes have disappeared with formation of lacunae. The tonofilaments aggregate into dyskeratotic material.

The dyskeratotic cells also show many keratinosomes and vacuoles, thus developing into typical corps rond. They are numerous in the granular layer, lying separately in the lacunae. Some contain large keratohyalin granules. In most of these cells the nucleus is no longer intact (Fig. 4). Grains are also present, mostly intralacunar, in the upper granular layer.

In the horny layer there are parakeratotic cells and there are cytolytic changes in many cells.

TREATMENT

All patients, except for case 6, were treated locally with 0·05–0·2 % vitamin A acid with favourable results in most of the patients. Permanent treatment was necessary since the lesions deteriorated when local treatment was stopped.

DISCUSSION

In two patients (case 1 and 3) the lesions followed the lines of Blaschko, the other cases being zosteriform (case 2), linear (case 5 and 6) and systematized (case 4 and 7). Similar cases with unilateral, systematized, linear or zosteriform lesions have been reported by several authors, many of which were brief reports of cases presented at clinical meetings (Table 1).

In most patients the individual lesions were keratotic follicular and non-follicular papules as in our patients 1–4. Several cases also showed verrucous lesions suggesting naevus verrucosus, naevus unius lateris or lichen striatus. Verrucous lesions were more common on the limbs as in our patients 5 and 6. In a small number of patients less typical lesions, psoriasiform or eczematous as in our case 7 or lichenoid and translucent papules, were present.

All patients showed the typical histological features of focal acantholytic dyskeratosis.

Electronmicroscopy has not been done before in this disorder. Our electronmicroscopic observations in one case showed abnormalities similar to classical Darier's disease.

Classification of these unilateral cases as a variant (forme fruste) of Darier's disease or as an epidermal naevus is still a point of discussion. The lack of family history and absence of other signs of Darier's disease in all reported patients as well as the distribution of the lesions in many patients are in favour of a naevoid origin. In this context Ackerman (1972) and Ackerman & Goldman (1974) postulated an analogy with epidermolytic hyperkeratosis. That disorder is seen as a generalized autosomal dominant inherited disease and furthermore as unrelated systematized naevoid lesions.

Part of the reason for the dispute on classification of the cases with unilateral Darier-like lesions in naevoid distribution may be due to the few cases reported who show bilateral asymmetical lesions without a family history or other findings suggestive of Darier's disease. Such patients have been reported by Chester & Brown (1959) and Gartmann (1968). We think that it is likely that both patients represent the bilateral variant of this naevus.

This distinct type of epidermal naevus for which we propose the name 'acantholytic dyskeratotic epidermal naevus', is perhaps not rare. Since 1969 we have seen biopsy specimens showing the histological features of focal acantholytic dyskeratosis in thirty patients, the clinical expression being classical Darier's disease in fourteen patients and acantholytic dyskeratotic epidermal naevus in seven patients. This type of epidermal naevus has some special features uncommon in other epithelial naevi.

TABLE I.

Author(s)	Sex/age	Age of onset	Site (R = right, L = left)
Ackerman (1972)	F, 18	14	R trunk, thigh
Anke (1938)	M, 34	33	R face, chest, lumbar region
Anderson (1948)	M, 53	38	R neck, trunk, ankle
Binnick & Fleischmajer (1977)	M, 18	18	R chest, lower back
Carrié (1936)	F, 11	Infancy	L abdomen
Costello (1961)	F, 25	8	L neck, back, chest
Demetree, Lang & St Clair (1979)	M, 3/12	0	L arm, shoulder, back
Goette (1973)	M, 42	30	L leg
Grupper & Cardinne (1974)	F, 64	49	R thigh
Hesbacher (1970)	F, 29	17	L back, arm, thigh, leg
Kellum & Haserick (1962)	M, 69	68	R calf, dorsum of foot
Kentsch & Nurnberger (1979)	F, 30	19	R chest, hip, leg
Kresbach (1966)	F, 82	42	R trunk
Leeming (1969)	F, 30	27	R leg, thigh, buttock, lower back
Lubritz & Marascalco (1972)	F, 61	Infancy	R trunk, leg
	F, 19	?	L neck, back
Niebauer & Raff (1970)	F, 37	27	R chest, leg
Piovano, Pippione & Sicuro (1971)	M, 21	Infancy	L chest, flank, abdomen
Robinson (1950)	F, 30	23	R thigh, leg
Sauer (1955)	M, 39	26	L (lower) chest
Snyder (1958)	M, 18	17	R chest, lower trunk, thigh, leg
Strani & Sartoris (1967)	M, 45	25	R foot
Swann (1971)	F, 73	?	L lower leg
Vero (1933)	M, 38	17	L arm, chest, abdomen, back
Verret *et al.* (1979)	F, 51	51	L leg, buttock
Present cases	M, 47	39	L hemithorax and upper arm
	M, 27	23	L shoulder and chest
	M, 58	53	R hemithorax and abdomen
	M, 46	24	R back
	F, 17	17	L leg
	M, 77	62	L leg
	M, 47	27	L lower arm

Delayed onset is frequently noted since the lesions started at an age of 20 years or later in about half of the patients, which is rather late for epidermal naevi as well as for Darier's disease. Sunlight and hot moist weather may provoke the condition as in other conditions characterized by focal acantholytic dyskeratosis. Furthermore it may improve considerably with topical vitamin A acid.

In our opinion the unilateral variant of acantholytic dyskeratotic epidermal naevus is a well established entity. The possibility of the existence of a bilateral variant of this naevus needs more study.

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Malignant fibrous histiocytoma arising in a smallpox vaccination scar

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SUMMARY

A case of malignant fibrous histiocytoma arising in a smallpox vaccination scar is reported.

It is well recognised that malignant change can occur in cutaneous scars. The development of malignant tumours at the site of previous smallpox vaccination has been occasionally reported. Marmelzat (1968) described squamous and basal cell carcinomas and malignant melanomas, developing at time intervals varying from a few months to over 40 years, following vaccination. We report a case of malignant fibrous histiocytoma arising in a smallpox vaccination scar.

CASE REPORT

A 60-year-old man was referred because of a swelling which had developed at the site of a smallpox vaccination scar on his right upper arm. The vaccination had been carried out during the Second World War. A small lump had been present since that time, but had increased in size over the previous five years. Examination revealed a firm round tumour at the location of the vaccination scar. No lymph nodes were palpable in the right axilla and clinically the lesion was considered to be a lipoma. The lesion was excised and primary closure accomplished.

Macroscopically the tumour measured 5 × 5 × 4 cm and was present in the dermis and subcutaneous tissue. The cut surface had a white lobulated appearance. Light microscopy showed that the tumour had a storiform pattern with foamy histiocytes, chronic inflammatory cells, bizarre mononuclear and multinucleate giant cells mixed with fibrous elements (Fig. 1). Only occasional mitotic figures were present.

The appearance was considered to be that of a malignant fibrous histiocytoma. This was confirmed by electron microscopy which showed a biphasic pattern with histiocytic and fibroblastic differentiation as described by Harris (1980). Most giant and atypical mononuclear cells showed numerous microvilli and occasional lysosomes indicating their histiocytic origin (Fig. 2).

No clinical recurrence has been observed to date (7 months).

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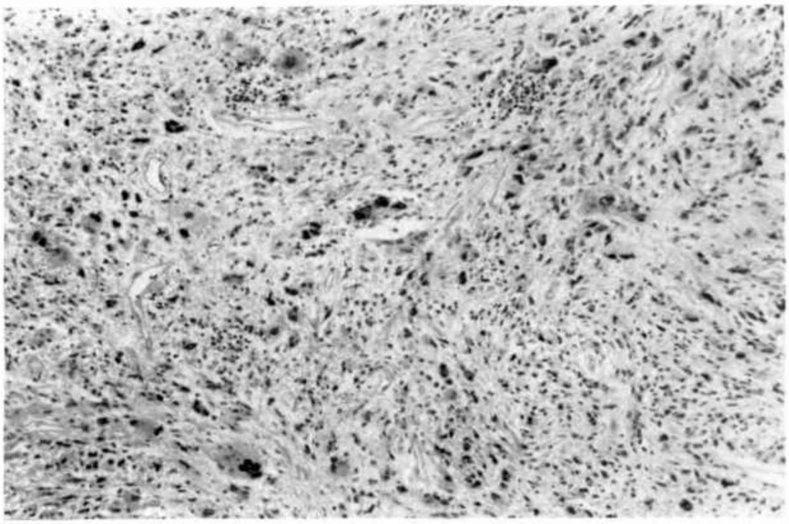

FIGURE 1. A storiform pattern is apparent and numerous multinucleate giant cells are present (H & E, × 42).

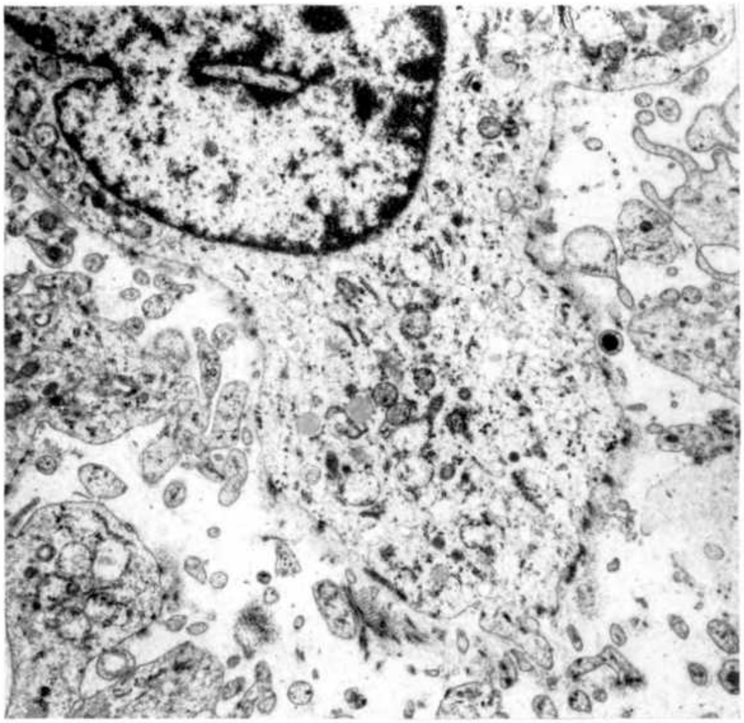

FIGURE 2. A histiocytic tumour cell with an irregular outline and showing microvilli, lipid droplets, sparse endoplasmic reticulum and occasional lysosomes (× 5,250).

DISCUSSION

Prior to the advent of electron microscopy, the diagnosis of 'soft tissue tumours' relied on the subjective impression of the pathologist and special stains. Nowadays by ultrastructural examination such tumours can be diagnosed with certainty in most instances. Malignant fibrous histiocytoma is now an accepted pathological entity and is characterized by cells with fibrohistiocytic differentiation (MacKenzie, 1975; Harris, 1980). Dermatofibroma protuberans is considered to be a low grade variant of the same tumour (MacKenzie, 1975).

Malignant fibrous histiocytoma has been described occurring in relation to bone infarcts (McCarthy, Matsuno & Dorfman, 1979) and it is possible that prolonged reparative processes, as may accompany bone infarction, may be an important factor in the pathogenesis of such tumours. Some authors have expressed the view that benign histiocytomas are not tumours at all, but represent a reactive proliferation of fibroblasts and histiocytes subsequent to trauma (Klaus & Winkelmann, 1966). It is therefore perhaps surprising that malignant fibrous histiocytoma does not occur more commonly in cutaneous scar tissue. Anaplastic fibrosarcomas have been described occurring in radiation and lupus vulgaris scars (MacKenzie, 1970). However, the only reported case similar to ours is that described by Coetzee (1964) when a sarcoma developed at the site of previous smallpox vaccination. Although the description conformed to dermatofibroma protuberans, ultrastructure studies were not performed.

ACKNOWLEDGMENTS

We are grateful to Mr D. Randall for permission to report this case.

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Brief Communication

Disappearance of epidermal Langerhans cells during PUVA therapy

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SUMMARY

The numbers and morphological appearance of epidermal Langerhans cells (LCs) were studied in twenty-five patients with psoriasis receiving treatment with 8-methoxypsoralen (8-MOP) and long wavelength UV irradiation (UV-A) (PUVA). After a single exposure, LCs showed loss of fine dendritic processes. Repeated treatments resulted in a reduction of the number of LCs from the mean pre-treatment value of 713/mm^2 to less than 60/mm^2 after seven treatments. The number of LCs remained low while treatment continued for up to 4 weeks. This finding may explain the impaired contact hypersensitivity observed in patients with psoriasis receiving PUVA therapy.

Patients with psoriasis receiving treatment with 8-methoxypsoralen (8-MOP) and long wavelength UV irradiation (UV-A) (PUVA) have diminished numbers and functions of circulating T lymphocytes (Morison *et al.*, 1979; Friedmann & Rogers, 1980) and reduced contact sensitivity to dinitrochlorobenzene (DNCB) (Strauss *et al.*, 1980; Moss, Friedmann & Shuster, 1981). Langerhans cells (LCs) are important for the induction of contact hypersensitivity (Streilein, Toews & Bergstresser, 1980) and they are destroyed by low doses of medium wavelength UV-B and high doses of UV-A (Aberer *et al.*, 1980). Since 8-MOP potentiates the actions of UV-A, the present study has examined the effects upon LCs of therapeutic doses of PUVA used in the treatment of psoriasis.

PATIENTS AND METHODS

Twenty-five psoriatic patients receiving PUVA treatment were studied. Paired observations before and during treatment were made on twelve patients and single observations at different times during treatment were made on the remainder. Langerhans cell density was measured in epidermal biopsies obtained from suction blisters raised on the anterior aspect of the upper arm. LCs were stained by the ATPase method (Juhlin & Shelley, 1977). The stained blister tops were spread on microscope slides and the density of LCs measured with a calibrated graticule at a magnification of × 400. At least 10 grids (about 50 cells per grid) were counted avoiding the openings of pilosebaceous follicles where LC density is increased.

RESULTS

LC density

Before treatment, the mean density of LCs in patients with psoriasis was 713 (s.e.mean 60)/mm^2 (Fig. 1). This was similar to that for six healthy controls: 730 (s.e.mean 60)/mm^2. As treatment pro-

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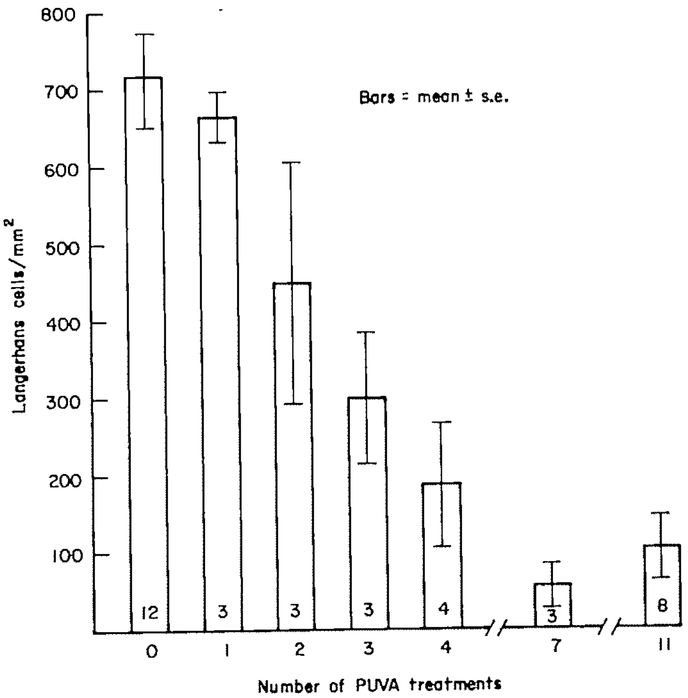

FIGURE 1. Effect of PUVA therapy on Langerhans cell density in psoriatic patients.

gressed LC density diminished. After three treatments (1 week) they were reduced by more than 50% to 300±28/mm² and after seven treatments there were only 56±29/mm² remaining (Fig. 1). With continued treatment, LC density remained low although in three patients who had become deeply tanned their numbers increased slightly.

LC morphology

The earliest change was a loss of the fine dendritic processes. This was sometimes visible after a single exposure to PUVA. As LC density diminished, there was also variation in their staining properties and faintly stained cells were seen adjacent to well stained cells.

After patients had been treated for 2–3 weeks (7–11 treatments), the few LCs that were seen were clearly of different morphology. They had a greatly elongated appearance with few coarse dendrites. They were well stained and were seen both in the basal layer and on its under side. They resembled the type II LCs described by Breathnach (1977).

DISCUSSION

The present observations show that LCs are sensitive to PUVA. A single treatment had only a slight effect but repeated exposures caused marked depletion of LC numbers and altered the morphology of those that remained. This altered morphology suggested that the cells had been damaged and it is therefore unlikely that numbers appeared reduced simply because of altered staining properties. In keeping with this, Aberer showed that both Ia antigens on LCs and their ability to stimulate a mixed lymphocyte reaction *in vitro* were destroyed by UV irradiation (Aberer *et al.*, 1980). The demonstration of LCs in and below the basal layer suggests either that they were on their way into or out of the epidermis or that LCs in that position were less affected by PUVA because of their depth or their state of maturity.

The depletion of LCs may explain the impairment of the induction phase of contact hypersensitivity which we have observed in patients with psoriasis receiving PUVA treatment (Moss *et al.*, 1981). Depletion of LCs by PUVA may also be important in its beneficial action on the cutaneous lymphoma mycosis fungoides since LCs may be involved in the control of lymphoid cell traffic in the skin (Streilein, 1978). Finally, if the LC is the epidermal target in chronic graft versus host disease PUVA may prove useful in treating or even preventing such reactions.

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Comment

THE CLINICAL RELEVANCE OF HEAT-STABLE, SHORT-TERM SENSITIZING ANAPHYLACTIC IgG ANTIBODIES (IgG S-TS) AND OF RELATED ACTIVITIES OF IgG_4 AND IgG_2

Most anaphylactic disorders, allergic rhinitis, asthma, allergic urticaria and angio-oedema, and anaphylactic gastro-enteritis, are mediated by IgE reaginic antibodies. An antibody mediating anaphylactic sensitivity binds to specific receptors on mast cell and basophil membranes by the Fc portion of the molecule, leaving the Fab portion free to react with antigen. IgE is the classic example of an anaphylactic antibody; its properties are well known.

(1) IgE normally occurs in very small amounts in the blood. However in passive sensitization tests, potent sera sensitize skin in high dilutions.

(2) It binds firmly to mast cells and basophils and is not easily eluted by prolonged washing of sensitized tissues *in vitro*.

(3) When the antibody is injected into the skin of a normal subject, as in passive cutaneous anaphylaxis or the Prausnitz-Kustner test, the site remains sensitized for 4 weeks or longer in man, and several days in the monkey.

(4) The ability of IgE to sensitize tissue is destroyed by heating at 56°C.

(5) The ability to sensitize is also destroyed by treatment with a chemical reducing agent.

ANAPHYLACTIC IgG (IgG S-TS) IN MAN

Many species of animals form IgE or IgE-like antibodies. Several species also form an IgG anaphylactic antibody which differs from IgE in being present in large enough amounts to be detected by agglutination or precipitation techniques, conferring sensitivity for hours or days only, and whose sensitizing activity is not destroyed by heat or, in most instances, by chemical reducing agents.

Man also forms an IgG antibody with anaphylactic properties and which may mediate anaphylactic disorders. This has been designated IgG S-TS (short-term sensitizing) because on passive transfer to normal skin the sensitivity persists for 2–4 h only.

The properties of IgG S-TS (Parish 1970, 1973, 1974, 1978) are:

(1) The antibody is present in pure fractions of IgG, uncontaminated by IgE.

(2) The IgG antibody binds weakly to tissues and is easily eluted *in vitro* by washing.

(3) When the antibody is injected into the skin of man or monkey, the site remains sensitized for 2–4 h only. The titre in passive cutaneous anaphylaxis tests is low, not greater than a 1/16 dilution. The weal and flare response closely resembles that mediated by IgE but is less intense and tends to be slightly longer in appearing and reaching its maximum size.

(4) IgG S-TS binds to human basophils *in vitro*, and it will partially block sensitization of skin *in vivo* by IgE for about 4 h. It apparently competes for the same, or more likely closely associated, receptor sites as IgE.

(5) The ability to sensitize tissue is unaffected by heating at 56°C or 60°C for up to 4 h.

(6) Sensitizing ability also resists chemical reduction with 0·1 M 2-mercapto-ethanol which inactivates IgE.

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(7) The skin responses *in vivo* in monkeys can be differentiated from immune complex-mediated (Arthus) reactions because depletion of complement and of neutrophils, both essential for Arthus reactions, do not diminish IgG S-TS responses. Some differences in the complement-depletion tests are attributed to technique and sources of reagents.

IMMUNO-ADSORBENT SEPARATION OF IgG S-TS AND IgE ACTIVITIES

In an examination of human antibodies for anaphylactic activity it is essential to determine that the activity is not due to contamination by IgE. The resistance of the sensitizing property to heat and to chemical reduction, and the short duration of passive sensitization, are very different from the properties of IgE.

In more critical tests the noninvolvement of IgE was confirmed by separating IgG from whole serum by two sequential fractionations on DEAE-cellulose to obtain a pure sample of IgG. This was treated with an insoluble anti-IgE immuno-adsorbent, shown capable of removing IgE reagin from other sera and serum fractions containing it. This treatment did not remove the anaphylactic antibody; the resulting preparation of electrophoretically medium to fast IgG contained the short-term sensitizing activity. Treatment of the fraction, or of whole serum, with anti-IgG Fc (γ determinant) resulted in much precipitation and loss of the sensitizing activity from the supernatant fluid (Parish 1973, 1974).

The non-involvement of IgE was confirmed by treating sera containing both short-term sensitizing and IgE antibodies to *Aspergillus fumigatus* with another anti-IgE immuno-adsorbent. This treatment removed the IgE antibodies but left the short-term sensitizing antibodies intact (Pepys *et al.*, 1979a). In a further test the IgG preparation containing IgG S-TS was injected into rabbits with Freund's adjuvant and the resulting rabbit antibody examined by radio-isotope binding methods to detect any impurity. No evidence of IgE or other serum proteins was detected in the original IgG fraction by this very discriminating method. Similar results were obtained by Bryant, Burns & Lazarus (1975) who found that the heat-stable anaphylactic IgG in asthmatic sera was not removed by anti-IgE, but was removed by treatment with an immuno-adsorbent column of anti-IgG. Furthermore, in a serum of a patient sensitive to protamine sulphate, the IgG anaphylactic antibody to protamine was removed from the serum by precipitation with anti-IgG, but not by anti-IgE antisera (Lakin *et al.*, 1978).

IgG S-TS tends to be found in sera with precipitating complement-fixing antibody to the test antigen, but it appears to be a special property of only a proportion of the total IgG because many sera with precipitins have no IgG S-TS activity as in the examples in Table 1.

Though IgG antibodies to foods occur frequently to a wide range of antigens, few of the antigens induce formation of precipitins, and the antibodies are detected by more discriminating techniques, agglutination, complement fixation, ELISA, modified RAST, or radio-immunoprecipitation in gels. Milk antigens not infrequently, and egg and wheat occasionally, induce formation of IgG precipitins. IgG S-TS antibodies have been detected to several milk antigens and to wheat and gluten fractions. IgE antibodies to these antigens may be found sometimes together with the IgG S-TS.

IN VITRO PASSIVE SENSITIZATION OF TISSUES AND BASOPHILS

IgE antibodies sensitize appropriate (mast cell-containing) tissues, e.g. lung and skin, *in vitro*. The tissues remain sensitized when washed to remove unbound antibody, and on subsequent treatment with antigen the amounts of mediators, e.g. histamine, released can be assayed as an indication of the degree of sensitization. Alternatively, tissues also containing smooth muscle, e.g. monkey ileum or

TABLE 1. Examples of sera with complement-activating precipitins without IgG S-TS activity

	Number of sera with			
Antigen	Precipitins	C. haem*	C1q†	S-TS
Milk	17	17	nd	0
Egg	6	6	6	0
Horse serum	28	28	28	0
Strep exotoxin	13	13	13	0
Aspergillus (Aspergilloma)	22	nd	nd	0
M. faeni (Farmers lung)	214	nd	189	0

* Haemolytic complement fixation test.
† C1q binding.
nd—Not done.

human bronchial strips, can be tested for contraction on antigenic challenge. IgG S-TS antibodies sensitize some samples of human skin and lung, but the amounts of mediators released are considerably less than released from tissues sensitized by IgE. Evidence of the weak binding of IgG S-TS to tissues was found in these tests, because sensitization was achieved more frequently if thicker than normal tissue preparations were used, or the washing of the sensitized tissue was reduced. It is of interest that IgG S-TS mediates release of both histamine and eosinophil chemotactic factor (ECF-A) from human skin indicating that skin test anaphylactic changes can to some extent be reproduced *in vitro* (Parish, 1974). In later tests (Parish, unpublished) other anaphylactic mediators were detected and the identity of the ECF-A confirmed. No slow reacting substance SRS-A was found in any sample.

IgG S-TS failed to show anaphylactic sensitization of monkey ileum or human bronchus *in vitro*, possibly because the antibody was too easily removed in the washing procedures.

Tests on human basophils indicated that there may be a critical number of IgG S-TS molecules, or critical spacing on the cell membrane, to achieve anaphylactic sensitization. IgE sensitizes human basophils *in vitro* to release histamine, and to shed granules, on subsequent treatment with anti-IgE antibody (anti-epsilon sera), or with the specific antigen. In contrast, IgG from IgG S-TS-containing preparations sensitizes basophils for histamine release on treatment with anti-IgG (anti-gamma) but not on treatment with the specific antigen. This critical test of antigen-induced anaphylactic activity has not so far been achieved despite many attempts. It is relevant that we have obtained the same results with IgG_4 preparations, which will sensitize basophils to react with anti-G_4 but not with antigen, as described later.

IgG S-TS IN CLINICAL DISORDERS

IgG S-TS, or heat-stable short-term sensitizing antibodies presumably IgG S-TS, have been reported in a wide range of clinical disorders, almost all of which are manifestations of anaphylactic sensitivity (Table 2). IgG S-TS antibodies occur most frequently in gastro-intestinal food allergy, and in asthma. The several antigens inducing asthma may be inhaled or ingested; inhaled antigens are common domestic environmental contaminants, or industrial dusts. It is particularly interesting that several microbial antigens induce formation of IgG S-TS antibodies, *Aspergillus fumigatus*, *Candida albicans*, *Streptococcus pyogenes* and tetanus toxoid.

TABLE 2. IgE and IgG S-TS. Comparison of sensitization of tissues for passive anaphylaxis

Test	Ability of IgE	Ability of IgG S-TS
P. K. man	+	+
PCA monkey	+	+
Human skin *in vitro*	+	±
Human lung *in vitro*	+	±
Human leukocytes	+	–*
Monkey leukocytes	+	–
SD† human bronchus	+	–
SD† monkey ileum	+	–

* IgG binds to basophils but no anaphylactic release of histamine with antigen.

† SD—Schultz Dale technique for anaphylactic contraction of passively sensitized strips of human bronchus or monkey ileum.

IgG S-TS antibodies have been detected in persons with generalized anaphylaxis following injection of tetanus toxoid (Parish, 1973, 1974), horse gamma globulin (Parish, 1973) and protamine sulphate (Lakin *et al.*, 1978). The responses to tetanus toxoid were mild, with generalized erythema, followed by pallor and mild shock. It is not surprising that injections of tetanus toxoid and horse gamma globulin therapeutic antisera, which induce formation of considerable amounts of IgG antibody, also give rise to hypersensitivity associated with IgG S-TS. The patient responding with dyspnoea, generalized urticaria and shock following an intravenous injection of protamine sulphate was found to have a heat-stable IgG anaphylactic antibody and no IgE to the same antigen (Lakin *et al.*, 1978). This IgG antibody was considered to be complement-dependent, as the anaphylactic response could not be induced in monkeys treated with cobra venom factor which depletes serum complement. However there are some technical difficulties with this procedure, and it has been reported that IgG S-TS is complement-dependent (Parish, 1970) and is not complement-dependent (Parish, 1973).

IgG S-TS is found in patients with food allergy, with gastro-intestinal changes or asthma. Milk is the most frequently detected allergen, especially β-lactoglobulin in persons with food allergic asthma, and this is the antigen most frequently inducing formation of IgE. Other milk antigens inducing formation of IgG S-TS antibodies are casein, α-lactalbumin and gamma globulin. In patients where gastro-intestinal manifestations (abdominal discomfort, pain, diarrhoea) start 3 h or more after ingesting the food allergen, IgG S-TS tends to be found in higher titres than in persons whose response starts within minutes of ingestion and who also have IgG S-TS.

There are several reports of IgG S-TS occurring in persons with asthma (Table 3). In some patients no clinical differences are detected from asthma in patients whose disorder is mediated by IgE. However in a comparison of fourteen atopic asthmatics with nine defined as cryptogenic (intrinsic) on the evidence of the 'late age of onset' of their asthma and negative prick test reactions to twenty-two routine common allergens, six atopic asthmatics had both short-term and IgE antibodies, and four of the patients with cryptogenic asthma had heat-stable short-term sensitizing antibodies and no IgE (Pepys *et al.*, 1979a). It would appear that in cryptogenic asthma IgG S-TS-like antibodies have an important role in the disease.

TABLE 3. Reports of clinical disorders attributed to IgG S-TS, or to heat-stable, short-term sensitizing antibody presumably IgG S-TS

Clinical disorder	Antigen	Reference
Food allergy	Milk especially β-lactoglobulin	Parish, 1970
Gastro-intestinal (some persons skin sensitive without allergy)	(Other food antigens in Brighton, 1980)	Parish, 1971a Parish, 1973 Brighton, 1980
Asthma	*Dermanyssus pteronyssinus*	Bryant, Burns & Lazarus, 1973 Bryant *et al.*, 1975
	House dust Grass pollen	Berry & Brighton, 1977
	Grass pollen Milk Egg Budgerigar	Pepys *et al.*, 1979a
	Platinum	Pepys *et al.*, 1979b
Allergic pulmonary aspergillosis	*Aspergillus fumigatus*	Pepys *et al.*, 1979a
Extrinsic allergic alveolitis	Avian antigens	Warren *et al.*, 1977
Anaphylaxis	Protamine sulphate	Lakin *et al.*, 1978 (this IgG was C-dependent)
Erythema with mild shock	Tetanus toxoid	Parish, 1973 Parish, 1974
Allergy to *Candida*	*Candida albicans*	Longbottom *et al.*, 1976
Vasculitis after streptococcal infection	*Stretococcus pyogenes*	Parish, 1971b
Serum sickness erythema, urticaria	Horse gamma globulin	Parish, 1973

Furthermore, in eleven nonatopic patients with asthma due to hypersensitivity to budgerigar serum antigen, allergic sensitivity was detected by intracutaneous injection of antigen, the prick tests being negative, by positive bronchial inhalation tests in all six of the patients examined by this procedure, and precipitins to budgerigar antigen in nine of eleven. IgG S-TS antibody was found in six of the group, being associated with IgE in four, and without IgE in two (Pepys *et al.*, 1979a).

The asthma related to avian antigens described above is relevant to another report in which extrinsic allergic alveolitis in six patients was also related to exposure to avian antigens, three of the six to budgerigar. IgG precipitins were found to several avian antigens and all six patients had an IgG S-TS-like antibody detected by P.K. tests on man (Warren, Cherniak & Tse, 1977).

On present evidence it cannot be stated categorically that IgG S-TS mediates the disorders in which it is detected. It certainly mediates the same anaphylactic changes in human or monkey skin mediated by IgE, though the response is weaker. It will weakly sensitize human lung and skin to antigenic challenge *in vitro*, and it binds to human basophils. It is very probable that IgG S-TS mediates some anaphylactic change in patients.

IgG S-TS not infrequently occurs concomitantly with IgE to the same antigen (Pepys *et al.*, 1979a; Brighton, 1980). In these patients it may well augment the activity of IgE, increasing the severity of the allergic responses.

IgG S-TS may occur in the absence of detectable IgE antibody to the antigen. In some of these patients, the response differs from that of a typical IgE mediated response, as in gastro-intestinal signs appearing 3 h or more after the food allergic patient has eaten the antigen. In such patients there is always the possibility that the tissues of the patient are sensitized by IgE and there is too little to detect in the serum. Another possibility is that IgG blocking antibodies may inhibit detection of IgE by RAST. This phenomenon was examined by Pepys *et al.* (1979a) who found that this did not apply to some sera containing IgG S-TS antibodies, and the sera were without IgE antibodies.

IgG S-TS AND SUBCLASSES OF IgG

The subclass of IgG S-TS has not been determined, but two IgG subclasses have been found to be associated with anaphylactic sensitivity, IgG_4 and IgG_2. Furthermore there are similarities in biological activity between IgG S-TS and IgG_4, but it has not been proven that IgG_4 has anaphylactic activity and is IgG S-TS.

IgG_4

Both IgG_4 and IgE are reported to be increased in amount in patients with atopic dermatitis (Shakib *et al.*, 1977), and in rhinitis, asthma or atopic dermatitis a correlation was found between IgE and IgG_4 antibodies to some allergens, though in others, e.g. guinea-pig dander, there were IgG_4 antibodies only (Bruynzeel & Berrens, 1979). Increases in total IgG_4 and IgE were found by Gwynn *et al.* (1978, 1979) to be related to the clinical picture. Both immunoglobulins were much increased in children with eczema, asthma and hayfever, but in asthmatic children without eczema an increase was sometimes detected in only one of the two immunoglobulins. Eighteen of 104 near relatives also had increased amounts of IgG_4 and though the relation between amounts of the immunoglobulins and disease was inconsistent, Gwynn *et al.* (1979) proposed that the same polygenic factors may control IgE and IgG_4 concentrations.

In allergy to other allergens, total and antigen-specific IgG_4 was found increased in patients allergic to tetanus toxoid (Parish, 1974), with asthma due to castor bean (Devey & Panzani, 1975) and with anaphylactic sensitivity to factor VIII in a haemophiliac patient (Shakib & Stanworth, 1979).

IgG S-TS and IgG_4 have some similarities in biological activity, but, as already stated, there is no conclusive evidence that IgG_4 is an anaphylactic antibody. Sera with increased amounts of total IgG_4 and containing IgG_4 antibody to the allergen to which the serum donor is allergic have frequently failed to sensitize monkey skin *in vivo*, or human tissues *in vitro*.

(1) IgG S-TS and IgG_4 specific antibodies occur in sera of patients with anaphylactic allergy. Both may occur together with IgE (references above), and some sera from patients allergic to tetanus toxoid contain both IgG S-TS activity and increased total and specific IgG_4 (Parish, 1974).

(2) IgG containing IgG S-TS sensitizes human basophils *in vitro* to release histamine on challenge with anti-IgG (anti-gamma) though no significant release of histamine occurred on challenge with antigen (Parish, 1973). Similarly preparations of IgG_4 from normal (non-myeloma) sera bind to basophils which release histamine on challenge with anti-G_4 (Table 4). However, no consistent release of histamine followed challenge with specific antigen. Myeloma IgG_4 has also been shown to bind to human leucocytes which released histamine on challenge with anti-G_4 (Vijay & Perelmutter, 1977).

The *in vitro* findings have a counterpart *in vivo*. Challenge of leukocytes from patients with allergic bronchopulmonary aspergillosis challenged with anti-IgG_4 released histamine, though the cells also responded to anti-IgG_2 and anti-IgG_3 (Assem & Turner-Warwick, 1976).

(3) In monkey passive cutaneous anaphylaxis IgG S-TS reduces or inhibits sensitization by IgE for up to 4 h (Parish, 1973). Similarly IgG_4 myeloma will block sensitization of monkey skin for 4 h. (Stanworth & Smith, 1973; Vijay & Perelmutter, 1977).

Further evidence of the blocking activity of IgG_4 for IgE has been obtained *in vitro* (Table 4).

TABLE 4. IgG_4 blocking IgE sensitization *in vitro*. Treatment of human leukocytes with IgG_4 followed by IgE antibody and by antigen

Subsequent treatment	% Histamine release				
	0 mg/ml IgG_4	0·1 mg/ml IgG_4	0·5 mg/ml IgG_4	1·0 mg/ml IgG_4	2·5 mg/ml IgG_4
Anti-IgG_4	1	3	14	21	23
IgE 1·2 μg/ml β-lact 10 μg/ml	32	38	30	21	6
IgE 1·9 μg/ml *A. fumigatus* 4 μg/ml	58	54	53	41	32

Suspensions of partially purified human basophils were treated with a range of concentrations of immuno-adsorbent purified IgG_4, free of all IgE, and washed. A sample of each preparation was tested with anti-IgG_4 to determine histamine release as evidence of IgG_4 binding. Of two other samples, one was treated with an IgE anti-β lactoglobulin (total IgE content 1·2 μg/ml), and the other with a potent IgE anti-aspergillus (total IgE content 1·9 μg/ml). After 1 h the cells were gently washed and challenged with the appropriate antigen. The reduced release of histamine, particularly with the weaker IgE antibody to β-lactoglobulin, indicated that the larger amounts of IgG_4 hindered sensitization by IgE. As a control, IgG_1 which did not sensitize basophils to release histamine with anti-IgG_1, also failed to reduce significantly IgE anaphylactic sensitization (Table 5).

TABLE 5. Treatment of human leukocytes with IgG_1 followed by IgE antibody and antigen

Subsequent treatment	% Histamine release				
	0 mg/ml IgG_1	0·1 mg/ml IgG_1	0·5 mg/ml IgG_1	1·0 mg/ml IgG_1	2·5 mg/ml IgG_1
Anti IgG_1	3	4	2	3	2
IgE 1·2 μg/ml β-lact 10 μg/ml	38				31
IgE 1·9 μg/ml *A. fumigatus* 4 μg/ml	52				56

(4) Patients with asthma, and whose sera contained IgG S-TS antibodies, were reported not to respond well to treatment with disodium cromoglycate (Bryant *et al.*, 1975). Similarly, 75% of asthmatic children with increased total amounts of IgG_4 did not respond satisfactorily when treated with the same drug, though most of those with increased total IgE only did so (Gwynn *et al.*, 1978).

There is little doubt that IgG_4 is a significant antibody to some allergens in anaphylactic hypersensitivity. It certainly binds to human leukocytes and predisposes them to degranulation and to histamine release on treatment with anti-IgG_4. The evidence indicates (to be published) that IgG_4 does not bind to the same receptors as IgE but to closely related receptors. The function of IgG_4 is not known, whether it is a blocking antibody, or whether it is an anaphylactic antibody. Our inconsistent results on antigen-induced histamine release from basophils could be interpreted that a critical number of IgG_4 molecules on the basophils, resulting in a particular distribution on the cell surface, would result in anaphylactic sensitivity to antigen. Fewer molecules may be without activity or augment IgE responses. More than the critical number of molecules may result in blocking both uptake of IgE and, at least *in vitro*, antigenic anaphylactic change. Such possibilities are being examined.

IgG$_2$

The other IgG subclass reported to have anaphylactic activity is IgG$_2$. Leukocytes of patients with allergic bronchopulmonary aspergillosis released significant amounts of histamine when treated with anti-IgG$_2$, though they also responded on challenge with anti-IgG$_3$ and anti-IgG$_4$ (Assem & Turner-Warwick, 1976). Furthermore, sera heated to destroy IgE cell-binding activity, passively sensitized human leukocytes to histamine release on subsequent treatment with anti-IgG$_2$, and also with the specific aspergillus antigen.

CONCLUSIONS

There is now much evidence that man may form an IgG antibody with weak anaphylactic activity. It differs from IgE in many of its properties, among which are that it sensitizes tissue for about 4 h only, the affinity for tissues is weak, the sensitizing activity is stable when heated at 56° or 60°C, and it resists chemical reduction.

The IgG subclass or subclasses of IgG S-TS activity are not known, but IgG S-TS and IgG$_4$ share several properties. Both will sensitize human basophils to challenge with anti-IgG or anti-IgG$_4$ respectively, both will hinder or inhibit IgE sensitization of skin for about 4 h, and asthmatic patients with IgG S-TS or with increased amounts of IgG$_4$ do not respond well to treatment with disodium cromoglycate.

IgG S-TS probably enhances the anaphylactic sensitivity of patients who also have IgE to the same antigen. It can probably mediate anaphylactic changes in some patients with little or no detectable IgE to the offending antigen. The clinical conditions most associated with IgG S-TS, are asthma, particularly cryptogenic (intrinsic) and asthma in nonatopic persons, food allergy, particularly if the signs are delayed some hours after taking the food, and allergies resulting from injected therapeutic substances, among which are tetanus toxoid and horse gamma globulin antisera.

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Book Reviews

Oral Manifestations of Systemic Disease. Edited by J.HAROLD JONES and DAVID K.MASON (1980). London: W.B.Saunders Company Ltd. Pp. 559. Price £32.50.

This is a large, well-produced, multi-author text with excellent black and white illustrations, a good index and many references—in most chapters up to 1977 and in some up to 1978. The contributors, who come from Britain (with Manchester and Glasgow heavily represented), U.S.A. and Denmark, have produced a readable text which should prove a valuable source of information on both the soft tissue and dental abnormalities in the mouth in a wide range of systemic diseases. Moreover, in this book the term 'systemic diseases' includes dermatoses which do not have any internal associations.

This volume is well worth a place on the shelves of all hospital departments of dermatology and although the editors are careful to point out that it is not a comprehensive work of reference for specialist stomatologists or dermatologists, both groups will surely use it for reference. Would-be purchasers of the book should, however, be warned that a considerable proportion of the text is taken up with short accounts of the general features of the disease concerned. These are similar in scope to those in a medium-sized undergraduate medical textbook and are clearly intended to provide necessary background for the dentist who is not medically qualified. In some cases this information takes up more space than the descriptions of the oral pathology. There is also some duplication and overlapping which could have been edited out—for example the two accounts of hyperparathyroidism—but in the first edition of a large text this is understandable and probably inevitable.

The dermatologist will find that about half of this book is potentially useful to him and will not, I suspect, wish to buy a personal copy—but he will undoubtedly want to have it on hand in his department.

S.O.B.ROBERTS

Oral Premalignancy (Proceedings of the First Dows Symposium). Edited by IAN C.MACKENZIE, ERIK DABELSTEEN and CHRISTOPHER A.SQUIER (1980). University of Iowa Press. Pp. 353. Price $32.50.

This book is a recording of the Proceedings of the First Dows Symposium held in the University of Iowa in 1977. There are nineteen contributors, mainly dental scientists and oral pathologists, from the U.S.A., Britain, Denmark and Canada.

The pages of this clearly written, massive literature review are packed full of information. The layout is excellent, the scope of each chapter is indicated at the beginning, and each chapter ends with a full list of references. There is no subject index for the entire book.

The first section deals with the clinical and histopathological concepts, the second with aetiology, the third with models for studying changes in oral premalignancy, the fourth with patterns of cell behaviour in normal and premalignant tissues, and the fifth on diagnosis and prognosis. The sections headed 'Discussion' which follow many of the chapters focus on dominant issues.

The book offers interesting reading with many new ideas, but at the same time reminds us that solutions are never as easy as they may seem. Obviously the reader's own interest would tend to colour his assessment of the relative value of the presentations. What the authors have mostly failed to do is to give guidance for further pursuance of research.

The text is supported by many excellent illustrations, photomicrographs, tables and charts. The great attraction of this book is the breadth of coverage and it provides an excellent source of interesting reading and valuable references for both clinician and pathologist alike.

B.E.D.COOKE

Cutaneous Aspects of Internal Disease. Edited by JEFFREY P.CALLEN (1981). Chicago & London: Yearbook Medical Publishers Inc. Pp. 682. Price: £66.00.

The author's self-confessed 'personal fascination with internal medicine and dermatology', admirable though it is, is not necessarily sufficient reason for producing a book on the subject. Quite apart from the question of whether it is a good book or not it seems sensible also to consider whether we needed another book on this subject, whether it has anything new or different to say, and at what part of the market it is directed.

The book is divided into six sections: rheumatology; vascular diseases; haematology and oncology; cardiopulmonary disorders; endocrinological and metabolic disorders; and miscellaneous disorders. Within this structure there are in all fifty-four chapters written by forty-five contributors. Some of Dr Callen's contributors, for example Sams and Voorhees, are world famous, much respected workers in their subjects. Others are less well known, in this country at least, and it is difficult therefore to know how authoritative their views are. Often their names do not appear in the lists of references at the ends of their chapters which indicates, unless they are excessively modest, that they are not particularly active in the fields about which they write. With regard to quality of information, it is usually easiest, if a little unfair, to judge a book such as this by the chapters that deal with one's own pet subjects and in some of these the information, though rarely incorrect, seems short on the facts and biased in the references.

The author's statement that several clinical entities can only be appreciated in full colour should in my opinion be expanded to all dermatological conditions. The few colour photographs in the book are excellent but the many black and white ones add nothing except to the expense.

Obviously the book occupies a place somewhat between the short paper-back and the dermatological 'bible'. Most people with an interest in dermatology will own or have access to both and I suspect will not pay £66 for this volume. The preface suggests 'the primary care physician and specialist in a broad range of clinical disciplines' will refer to it, but in this country they too use paperbacks and 'bibles' even more often it seems, than their dermatological colleagues.

JANET M.MARKS

Woods Injurious to Human Health. BJÖRN M.HAUSEN (1981). Berlin, New York: De Gruyter. Pp. 189. Price DM69.00.

In 1973 Dr Björn Hausen of Hamburg published in German a compact but comprehensive monograph on woods which present health hazards. This useful work was very well received and there has been an insistent demand for an English edition. This the author has now provided but he has taken the opportunity of enlarging the book and including the publications of the past eight years.

After short introductory chapters on the incidence, clinical features and immunological aspects of reactions to wood, and on wood chemistry, the greater part of the book provides a systematic survey of the offending species of trees, covering the technical and botanical as well as the medical literature. The last two chapters are concerned with asthma and with adenocarcinoma of the nasopharynx. The indices are exhaustive; one of them lists all the indigenous and trade names by which timbers are known and this greatly increases the value of the book since such names are usually the only ones known to the potential patients who are handling the woods.

This small book contains in a readily accessible form a great deal of information widely scattered through the literature of several disciplines and it deserves a place in the library of every department of dermatology which is called upon to investigate cases of wood dermatitis.

ARTHUR ROOK

A Colour Atlas of Nutritional Disorders. DONALD S.MCLAREN (1981). London: Wolfe Medical Publications. Pp. 109. Price £15.00.

This book consists of 288 illustrations, mostly in colour, beautifully produced on high quality art paper. As with the other volumes in this series the standard of reproduction of the photographs and indeed, all technical aspects of the book are very high. The subject matter crosses many medical boundaries including dermatology, surgery, radiology, paediatrics and of course nutritional medicine. It provides fascinating browsing for any medical reader and emphasizes the great sadness of today's world in which several billion pounds can be spent on a new airport for Saudi Arabia and greater sums on an American space shuttle. Perhaps its greatest value lies in a propaganda exercise for a relatively unconcerned medical profession.

A relatively small proportion of the illustrations are of specific dermatological interest and even these seemed to be aimed at a general audience rather than the specialist. Few dermatologists will be familiar with the 'signa da bandera' dramatically illustrated as broad light and dark alternating bands in the hair corresponding to periods of poor and better nutrition, but all would surely protest at the legend to 'hypercarotenaemia of the sole of the foot' that 'the staining is usually heaviest on the palms and soles due to the secretion of carotenoids by sebaceous glands heavily concentrated in these areas', when these are the very areas from which pilosebaceous follicles are absent.

I concluded that this volume would be a popular addition to general medical libraries but not, I think, to the dermatologists' personal or departmental collections.

W.A.D.GRIFFITHS

British Journal of Dermatology (1981) **105**, 237.

News and Notices

Investigative Group Meeting, BAD, 27 and 28 January 1982

The Annual Meeting of the Investigative Group of the British Association of Dermatologists will be held in Bristol on Wednesday and Thursday, 27 and 28 January 1982, and will be followed by a meeting of The British Society for Dermatopathology. The Investigative Group meeting will include lunch-time workshop sessions and a half-day symposium on collagen. A travelling scholarship of £200 to enable the recipient to attend the E.S.D.R. annual meeting will be given to the presenter of the best abstract submitted. Those who wish their abstracts to be considered for the scholarship should include a full curriculum vitae with their submitted abstracts, which may be for either oral or poster communications.

Nine copies of the abstract should be sent, typed in single spacing on one side of a sheet of A5 paper (105 × 148 mm). This abstract must contain a statement of the purpose of the study, methods used, and summary of the results. Please underline the name of the author who will present the paper.

Please forward abstracts to: Professor R.M.MacKie, University Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU, not later than 31 October 1981. (Abstracts not received by this date will not be considered.)

Full details of the meeting will be circularized to members of the BAD Investigative Group later in the autumn. Others interested should contact Professor R.M.MacKie. The meeting is open to clinical and non-clinical colleagues.

Lasers and the Skin

A Symposium on Saturday, 19 September 1981, will discuss the interaction of argon and dye lasers with skin and the treatment of port wine stains and other cutaneous lesions.

Further information may be obtained from: Dr Kenneth A.Arndt MD, 330 Brookline Avenue, Boston, MA022.5, U.S.A.

International Federation of Societies of Cosmetic Chemists

The 12th I.F.S.C.C. Congress will take place in Paris from 13 to 17 September 1982. Abstracts (a maximum of 400 words for papers and posters) must be submitted in English before 30 November 1981 to Société Française de Cosmétologie, 44 rue du 22 Septembre, 92400 Courbevoie, France.

Ninth Annual Paediatric Dermatology Seminar

This will be held in Miami Beach, Florida, from 25 to 28 February 1982. The Seminar fee will be $190. For further information contact: Dr Guinter Kahn, 16800 NW 2 Ave., Miami, Florida 33169, U.S.A.

Paediatric Dermatology Course

A course for Senior Registrars and recently appointed Consultants will be held in Liverpool on 12 and 13 November 1981. The course will be limited to twelve participants. For further information contact: Dr J.L.Verbov, Post Graduate Medical Dean's Office, Duncan Building, University of Liverpool, Liverpool L69 3BX.

American Society for Clinical Pharmacology and Therapeutics

The biannual Dermatology and Allergy Section will be at Disney World, Lake Buena Vista, Florida on 17–20 March 1982. Meeting and membership information is obtained from Mrs E.Galasso, ASCPT, 1718 Gallagher Rd, Norristown, PA 19401, U.S.A.

Abstracts should be mailed by 1 September 1981 to Howard Maibach, M.D., University of California, Medical Center, 400 Parnassus Ave., Room A-342, San Francisco, CA 94143, U.S.A.

The Royal College of Physicians, London

The following dermatologists have recently been elected to The Fellowship of the Royal College of Physicians, London: Dr J.A.Cotterill, Dr M.G.C.Dahl, Dr N.E.Jensen, Dr P.E.Kilby, Dr A.McDonald, Dr F.M.Pope and Dr C.A. Ramsay.

APPOINTMENTS

Edinburgh

Dr R.St C.Barnetson has beeen appointed to the post of Consultant Dermatologist and part-time Senior Lecturer in the Royal Infirmary, Edinburgh.

He qualified in Edinburgh in 1964 and following his house jobs in the Royal Infirmary, served abroad in the RAMC. He returned to Edinburgh in 1970 as Registrar in Dermatology, passing the M.R.C.P. (U.K.) in 1971. In 1974 he was appointed Clinical Research Physician at the M.R.C. Leprosy Project in Addis Ababa, Ethiopia, and also honorary Assistant Professor in the Department of Medicine, National University, Addis Ababa. In 1976 he returned to Edinburgh as Lecturer in Dermatology where he completed his MD thesis in 1977 on immunological reactions in leprosy. His main interests are tropical dermatology and the immunology of skin disease, and he has written numerous papers on the subjects of leprosy, cutaneous leishmaniasis, fungal infections, atopic eczema and dermatitis herpetiformis.

Dyfed Health Authority

Dr Ian George Ralfs has been appointed Consultant Dermatologist to the Dyfed Health Authority. Dr Ralfs qualified M.B., Ch.B. (Liverpool) in 1970, and became M.R.C.P. (U.K.) in 1974. After this he became Wellcome Training Fellow in Dermatology and Immunology, obtaining M.Sc. (Birmingham) in Immunology in 1976. He was appointed Senior Registrar in Dermatology in Oxford in 1977.

His research interests have centred on alopecia areata and epidermal cell kinetics.

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Experimental Skin Grafts and Transplantation Immunity

A Recapitulation

1979. 60 figures. XIX, 192 pages
Cloth DM 62,–; approx. US $ 29.60; approx. £ 13.00
ISBN 3-540-90425-5

O. Braun-Falco, H. Goldschmidt, S. Lukacs

Dermatologic Radiotherapy

1976. 48 figures including 16 color plates. XIV, 154 pages
Cloth DM 35,40; approx. US $ 16.90; approx. £ 7.45
ISBN 3-540-90198-8

V. M. Der Kaloustian, A. K. Kurban

Genetic Diseases of the Skin

With a Foreword by F. Clarke Fraser
1979. 441 figures, 17 tables.
XIII, 339 pages
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Diagnosis and Therapy of Porphyrias and Lead Intoxication

International Symposium Clinical Biochemistry
Editor: M. Doss
1978. 107 figures, 70 tables.
XIX, 310 pages
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D. Fanta

Hormone Therapy of Acne

Clinical and Experimental Principles

1980. 25 figures, 21 tables.
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Wien-New York: Springer-Verlag
ISBN 3-211-81586-4

S. Fregert, H. J. Bandmann

Patch Testing

On behalf of the International Contact Dermatitis Research Group
With contributions by numerous experts
1975. 3 figures, 17 tables. V, 78 pages
DM 21,–; approx. US $ 10.00; approx. £ 4.40
ISBN 3-540-07229-2

J. Petres, M. Hundeiker

Dermatosurgery

With a Foreword by W. K. Kalkoff
Translation from the German
1978. 112 figures. XVII, 152 pages
Cloth DM 47,–; approx. US $ 22.40; approx. £ 9.90
ISBN 3-540-90296-1

Physical Modalities in Dermatologic Therapy

Radiotherapy - Electrosurgery - Phototherapy - Cryosurgery

Editor: H. Goldschmidt
1978. 317 figures (16 in color), 62 tables. XV, 290 pages
Cloth DM 92,–; approx. US $ 43.80; approx. £ 19.35
ISBN 3-540-90267-8

G. Plewig, A. M. Kligman

Acne

Morphogenesis and Treatment

1975. 110 plates, mostly in color.
XII, 333 pages
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Retinoids

Advances in Basic Research and Therapy
(Proceedings of the International Dermatology Symposium, Berlin, October 13–15, 1980)
Editors: C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, R. Schuppli
1981. 215 figures, 143 tables.
Approx. 550 pages
ISBN 3-540-10673-1
In preparation

B. Schaumann, M. Alter

Dermatoglyphics in Medical Disorders

1976. 74 figures, 51 tables.
XI, 258 pages
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Skin Microbiology: Relevance to Clinical Infection

Editors: H. Maibach, R. Aly
1981. Approx. 120 figures.
Approx. 385 pages
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In preparation

Springer-Verlag
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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldly names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 105, Number 2, August 1981

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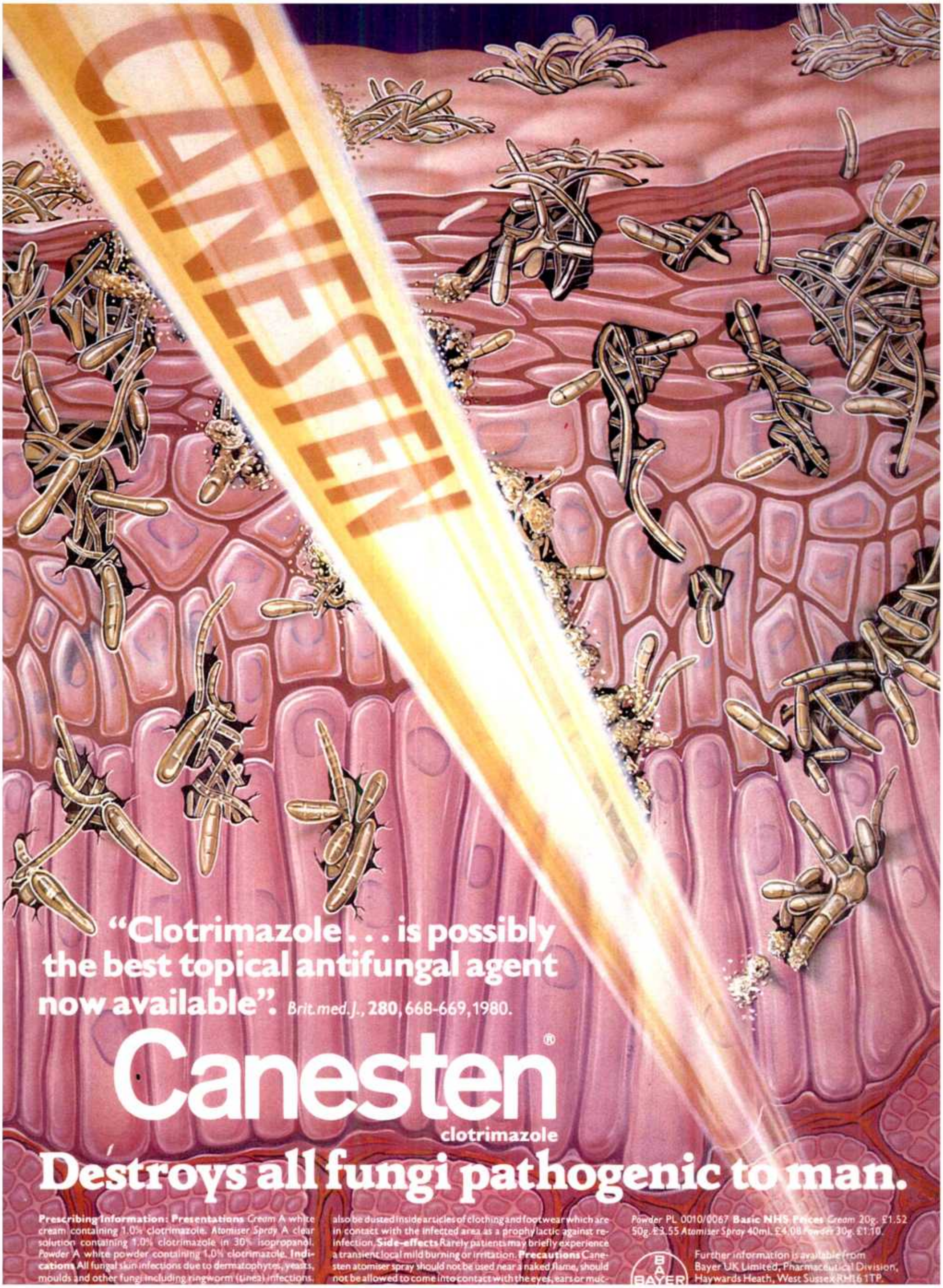
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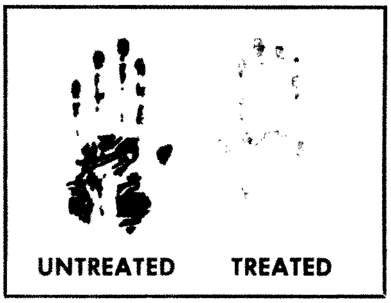

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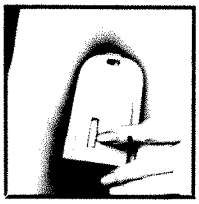

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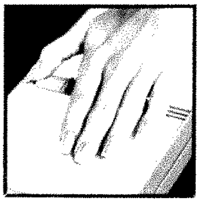

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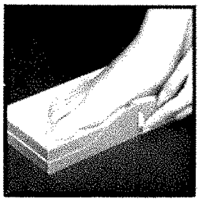

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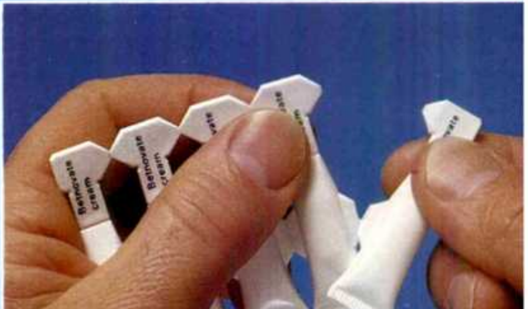

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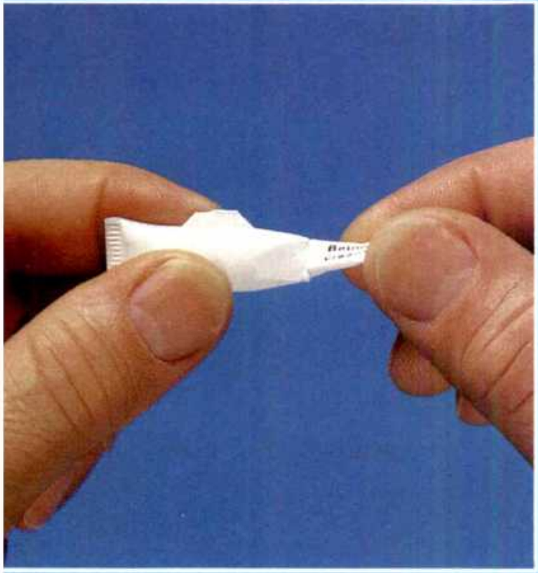

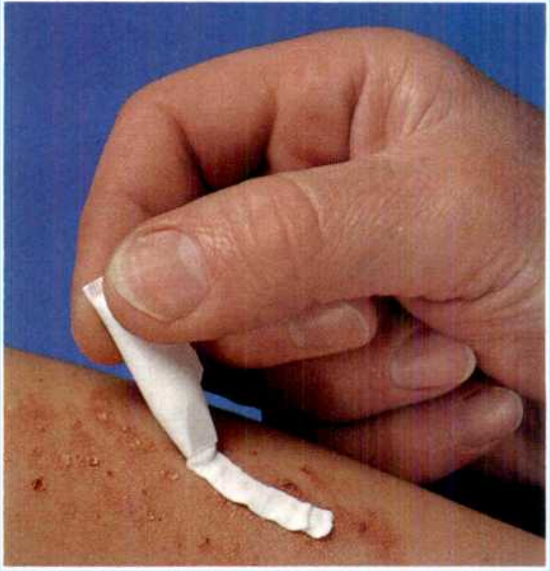

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Current Problems in Dermatology, Vol. 10
Series Editor: J.W.H. Mali, Nijmegen
ISSN 0070–2064

Biochemistry of Normal and Abnormal Epidermal Differentiation

Proceedings of the U.S.-Japan Seminar,
Boyne Mountain Lodge, Boyne Falls, Michigan,
July/August 1979

Editors: I.A. Bernstein, Ann Arbor, Mich., and
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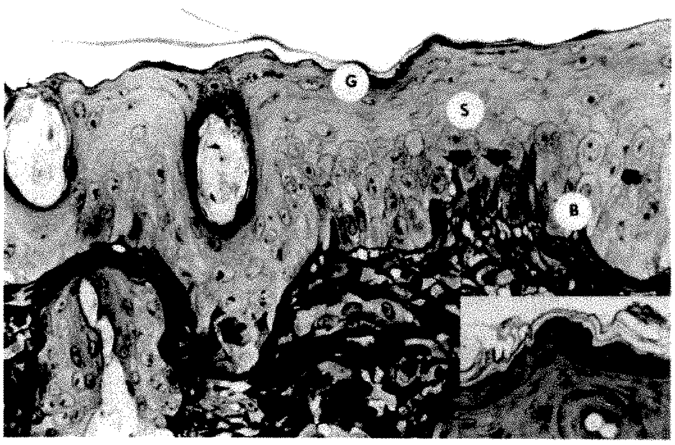
Fields of Interest: Dermatology; Biochemistry, Enzymology, Immunology, Molecular Biology



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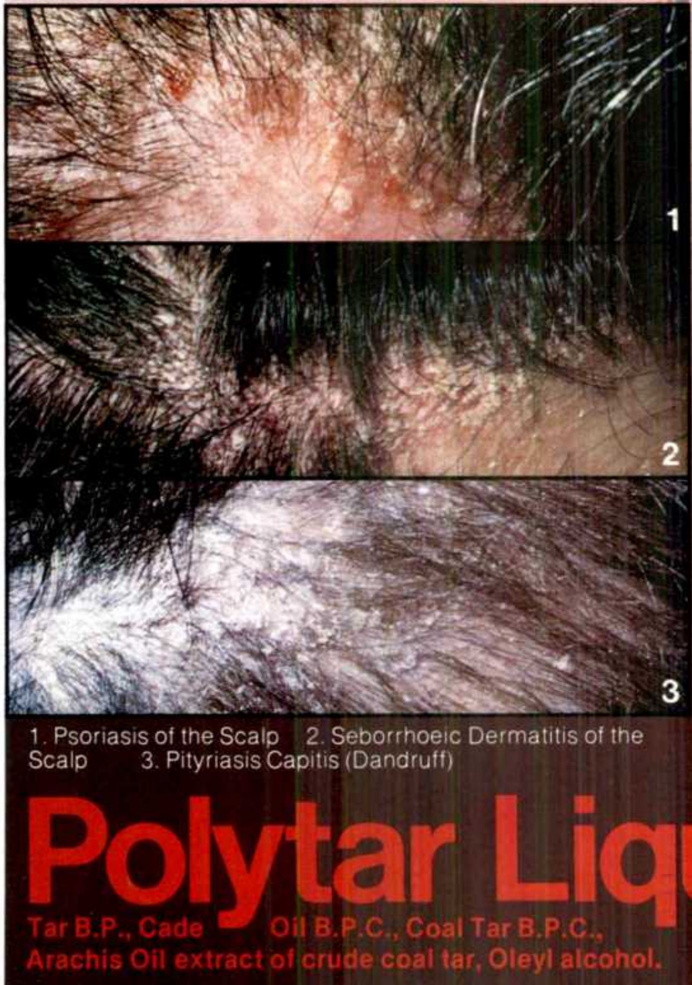

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Clinical and Laboratory Investigations

Circumscribed myxoedema of lichen myxoedematosus as a sign of faulty formation of the proteoglycan macromolecule

HIDEFUMI MAEDA, HIDEKAZU ISHIKAWA AND
SHIGEO OHTA

Department of Dermatology, Gunma University, School of Medicine, Maebashi, Japan

Accepted for publication 7 November 1980

SUMMARY

Using a new method devised by our laboratory, the ultrastructure of dermal glycosaminoglycan in an involved area of lichen myxoedematosus was examined.

Although histochemical and biochemical studies have indicated simply an accumulated deposition of hyaluronic acid in the lesion, the glycosaminoglycan ultrastructure within it was distinctly different from that in normal skin. The glycosaminoglycan structure of normal skin was similar to the proteoglycan aggregate model described by Rosenberg (1975). As confirmed by the enzymatic digestion procedure, it represents the ultrastructure of hyaluronic acid bound to glycosaminoglycans such as dermatan sulphate or chondroitin sulphate. In contrast, hyaluronic acid filaments observed in lesions of lichen myxoedematosus contained no glycosaminoglycan subunits.

Clinically there are two kinds of circumscribed myxoedema. Pretibial myxoedema appears as a skin symptom of Graves' disease, while the second type, lichen myxoedematosus or papular mucinosis, develops in patients without any thyroid disturbance. Circumscribed myxoedema of either type is characterized by localized deposition of hyaluronic acid in the corium (Sisson, 1960; Rudner, Mehregan & Pinkus, 1960; Takama, Ohashi & Iwata, 1976). Hyaluronic acid (HA) is known to play an essential role in the control of tissue fluid because of its strong hydropic activity (Preston, Davies & Ogston, 1965). Recent progress in biochemical analysis has indicated that HA is bound to many molecules of proteoglycan (glycosaminoglycan-protein complex) stabilized by link proteins (Hardingham & Muir, 1972, 1973; Hascall & Heinegard, 1974; Heinegard & Hascall, 1974). Rosenberg, Hellman & Klenschmidt (1970) reported the ultrastructure of proteoglycan extracted from bovine articular cartilage, and later, based upon his own and other investigations, he outlined a model for HA bound to proteoglycan subunits (proteoglycan aggregate) (Rosenberg, 1975). Although such a

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structure for proteoglycan has not been demonstrated hitherto in tissue specimens, including skin, we have previously suggested that ruthenium red staining by Luft's method indicated that the HA ultrastructure of the involved skin of lichen myxoedematosus or pretibial myxoedema might differ from that of normal skin (Ishikawa, Suzuki & Saito, 1976). Our recent investigations have made possible a more evident demonstration of the ruthenium red-positive ultrastructure (Ishikawa *et al.*, 1979). In the present paper, we provide evidence that the proteoglycan aggregate-like structure is also present in skin and that circumscribed myxoedema, at least of lichen myxoedematosus, appears to have a defect in the proteoglycan macromolecule.

MATERIAL AND METHODS

Biopsy specimens of involved back skin of two male patients (41 and 71 years old) with lichen myxoedematosus were used for the present study. Site-matched skin biopsies of five normal persons aged between 14 and 37 years and ten patients with systemic and localized scleroderma, or scleroedema of Buschke, aged between 21 and 72 years served as the controls.

The glycosaminoglycan ultrastructure of bovine articular cartilage was also compared with that of these skin samples. The skin specimens were divided for morphological and biochemical examination.

(1) For morphological study, the skin was fixed overnight at 4°C in a formol-ethanol (10:90) solution containing 1% (w/v) cetyltrimethylammonium bromide, and then divided into pieces for simultaneous light and electron microscopic examinations. For histological examination, after dehydration through a series of ethanol solutions, the skin specimens were finally embedded in paraffin. The sections were stained with HE, van Gieson, Weigert, alcian blue, or PAS. Histochemical identification of the alcian blue-positive glycosaminoglycan (GAG) was done by an enzymatic digestion procedure, using streptomyces- or testicular-hyaluronidase and chondroitinase AC, or ABC (Ishikawa & Mori, 1973). Ultrastructural demonstration of skin GAG was carried out according to the method previously described (Ishikawa *et al.*, 1979): Pieces of fixed skin specimens were treated with a series of decreasing concentrations of ethanol solutions ending with water. Then the tissue GAG was digested with previously described variable enzymes, including the enzymes used for the histochemical study. Thereafter, the skin specimens were post-fixed in 0·1 M phosphate buffer (pH 7·4) containing 0·05% ruthenium red and 2% OsO_4, followed by dehydration in ethanol and embedding in Epon 812. Ultrathin sections were observed with the JEM-T8 or JEM-100C electron microscope. In order to avoid a false positive reaction with ruthenium red staining, the specimen was first observed without contrast staining with uranyl acetate-lead citrate. The above electron microscopic procedure is depicted in Fig. 1. Enzymatic digestion of the GAG was carried out at 37°C for 30 min in one of the

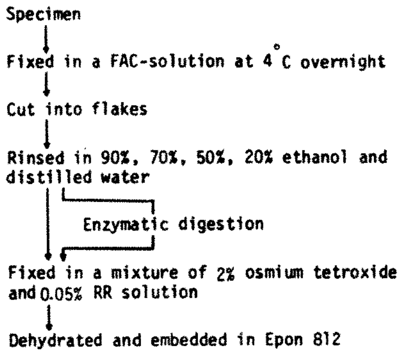
Specimen
Fixed in a FAC-solution at 4°C overnight
Cut into flakes
Rinsed in 90%, 70%, 50%, 20% ethanol and distilled water
Enzymatic digestion
Fixed in a mixture of 2% osmium tetroxide and 0.05% RR solution
Dehydrated and embedded in Epon 812

FIGURE 1. Method for the ultrastructural demonstration of dermal glycosaminoglycan.

following solutions: hyaluronidase digestion in 0·015 M acetic acid buffer (pH 5·4) containing 0·85% NaCl, and 150 TRU/ml streptomyces hyaluronidase (Amano Seiyaku, Japan) or 300 NFU testis hyaluronidase (Sigma); digestion with chondroitinase in 0·1 M tris-HCl buffer (pH 8·0 and 8·7) containing 0·8 U; 2 U/ml chondroitinase AC or 0·8 U/ml chondroitinase ABC (Seikagaku Kogyo, Japan); pronase digestion in 0·1 M phosphate buffer (pH 7·4) containing 100 tyrosine U/ml pronase E (Kaken Kagaku, Japan). An incubation with the buffer alone served as the control. Further, proteinase contamination in chondroitinase ABC or AC was checked using an isotope-labelled protein. The labelled protein was obtained from 3T3 BALB/c mouse fibroblasts cultured with [^{3}H]-proline. Chondroitinase ABC (lot:E9904) or AC (lot:E9Y01) was incubated at 37°C for 14 h in an assay mixture containing the [^{3}H]-protein (100,000 c.p.m.) and 0·1 M tris-HCl buffer (pH 8·0 and 8·7). When necessary, 5 mM benzamidine hydrochloride, 0·1 M 6-amino-*n*-caproic acid, and 0·04 M EDTA were added to the solution as proteinase inhibitors, a preparation adopted by Oegema, Hascall & Eisenstein (1979) in the isolation of aorta proteoglycan. After the incubation, the assay solution was deproteinized with 10% (final) TCA. Then the radioactivity of the supernatant was counted, and compared with the result of a control assay mixture without chondroitinase to determine the radioactivity of [^{3}H]-protein digested by the enzyme. The proteinase activity was expressed as pronase EU with the corresponding proteolytic activity estimated in a similar way. In the proteolytic activity determination of pronase, an equal amount of bovine serum albumin with that added in commercial chondroitinase was added to the assay mixture. The proteinase activity of 1 unit of chondroitinase ABC or AC at pH 8·0 or 8·7 was found to be less than 3×10^{-5} tyrosine U of pronase E. In addition, proteinase activity was negligible in the presence of the proteinase inhibitors. Therefore, for the present investigation, the incubation of skin specimens with chondroitinase was carried out with the inhibitors. Furthermore skin specimens were incubated with 0·1 M tris-HCl buffer (pH 8·0 and 8·7) containing 3×10^{-4} U/ml pronase E, as a control for proteolytic activity in chondroitinase.

(2) Biochemical assay: isolation of dermal GAG and determination of the total content as uronic acid as well as the distribution pattern of the constituent GAG were done by methods described previously (Ishikawa & Horiuchi, 1975).

RESULTS

Histochemistry

Myxoedema areas, which were arranged nodularly in the corium, revealed a rather weak positive reaction with alcian blue staining, in good agreement with accepted data. They were completely digested by streptomyces hyaluronidase.

Biochemical analysis

In one patient examined, the total GAG content as uronic acid (3·4 mg/g d.w.) was elevated as compared to that of normal skin (Ishikawa & Horiuchi, 1975). The GAG consisted of hyaluronic acid (90% of the total) and dermatan sulphate. This result accords very well with the histochemical findings, indicating increased HA deposition in the lesion.

Electron microscopy

The GAG ultrastructure of the involved skin area, as demonstrated by our method, showed strikingly different features from those of the normal skin. In the interfibrillar matrix of normal skin, we found long, rather straight filaments of about 3·0–7·0 nm in thickness. Attaching to them were many short, irregularly curled filaments of varying lengths (Fig. 2). This structure, at a glance, recalled the proteoglycan aggregate model of Rosenberg. In fact, the intervals of the short filaments arising from the long

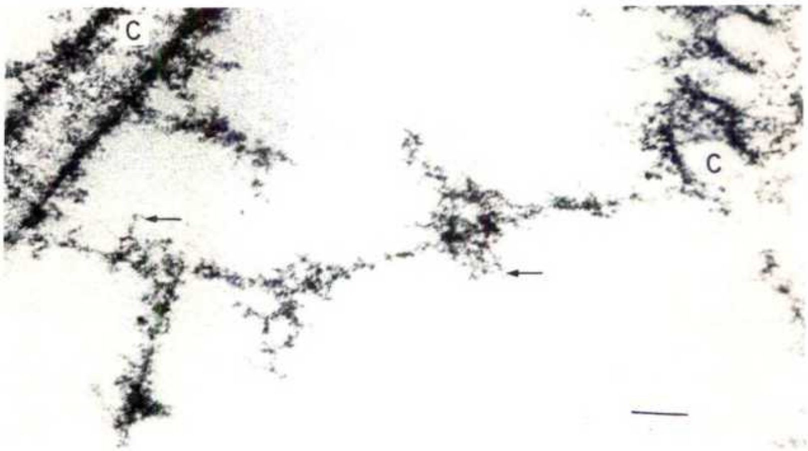

FIGURE 2. Electron micrograph showing a proteoglycan aggregate-like structure in the interfibrillar matrix of normal skin. The long filaments, with short side-filaments (arrow), are bridged between collagen fibrils (C). The bar represents 100 nm (× 76,800).

TABLE 1. Enzymatic digestion of the glycosaminoglycan structure in normal skin

Enzyme	GAG to be* digested	Long filaments	Short side-filaments
Streptomyces hyaluronidase	HA	+	+†
Testicular hyaluronidase	HA, Ch, Chs	+	+(†)
Chondroitinase ABC with proteinase inhibitors:			
at pH 8·0	HA, Ch, ChS, DS	+	+(†)
at pH 8·7	Ch, ChS, DS	−	+
Chondroitinase AC with proteinase inhibitors	HA, Ch, ChS	+	+(†)
Pronase		−	+
Buffer solutions alone, or 3×10^{-4} U/ml pronase‡		−	−
	Conclusion	HA	DS, ChS (or Ch) and protein

HA: hyaluronic acid; Ch: chondroitin; ChS: chondroitin sulphate; DS: dermatan sulphate.
+ : digested; − : not digested.

* Confirmed on cellulose acetate membrane electrophoresis.

† Pseudopositive reaction due to digestion of long filaments is hypothesized; it may be so also in (†).

‡ Control of proteinase activity of chondroitinase ABC or AC.

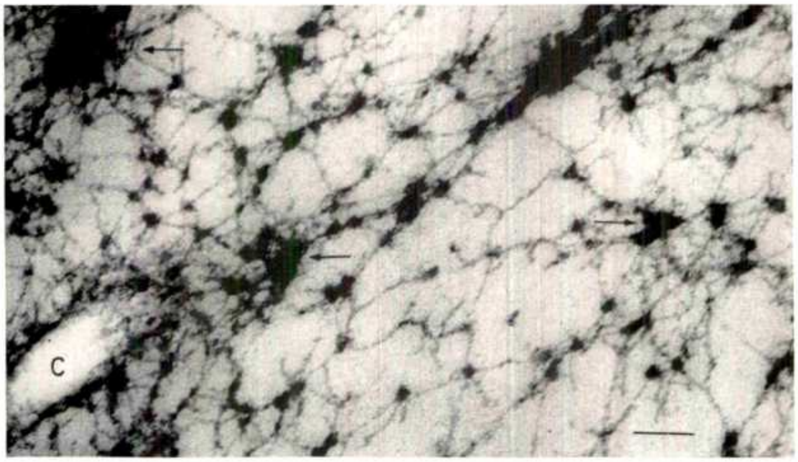

FIGURE 3. Electron micrograph showing fine networks of long filaments containing no side-filaments in lichen myxoedematosus. Arrows indicate aggregation granules (× 76,800).

filament were about 17·0–40·0 nm wide, very similar to those of the proteoglycan aggregate (Rosenberg, Hallman & Kleinschmidt, 1975; Heinegard, Lohmander & Thyberg, 1978).

Furthermore, the GAG structure of bovine articular cartilage resembled that of the skin, although the long filaments of the cartilage showed an extensive fine network. The results of enzymatic digestion of the above skin structure are summarized in Table I. The long filaments were completely digested by hyaluronidase, chondroitinase AC and ABC (pH 8·0), but not by either chondroitinase ABC at pH 8·7 or protease (pronase). On the other hand, the short side-chains were digested by chondroitinase ABC at pH 8·7 and pronase as well as by the above enzymes. Streptomyces hyaluronidase digests HA alone. Testicular hyaluronidase, chondroitinase AC and ABC (pH 8·0) can digest HA in addition to sulphated GAG, while chondroitinase ABC at pH 8·7 as well as pronase is not able to digest HA (Yamagata *et al.*, 1968). Chondroitinase ABC at pH 8·7 and pronase digest chondroitin sulphate A, C or dermatan sulphate and protein, respectively. The fact that the short filaments were digested by protease could be explained by either digestion of the core protein of proteoglycan or degradation of link protein. The fact that the short filaments also disappeared after digestion with streptomyces hyaluronidase could be interpreted as pseudopositivity based on a loss of stability due to digestion of the long filaments connected with the short filaments and this phenomenon might be considered also in an incubation with other HA-digestible enzymes. To summarize, the long filaments represent the ultrastructure of HA, and the short filaments may be composed of either dermatan sulphate or chondroitin sulphate-proteoglycan. The skin GAG structure of patients with scleroderma or scleredema and its behaviour after enzymatic digestion were similar to those of normal skin. On the other hand, the skin lesions of lichen myxoedematosus were enriched in long filaments which were inclined to form fine networks. However, we failed to see any short side-filaments attached to the long filaments (Fig. 3). Large aggregation granules were also observed. The behaviour of the observed filaments and granules in enzymatic digestion was similar to that of the long filaments in normal skin.

DISCUSSION

In previous electron microscopic studies, the ultrastructure of dermal GAG has been described as 'thread-like or lattice-like microfibrils' (Kobayashi, Danielson & Asboe-Hansen, 1971) or networks composed of fine filaments and granules (Kajikawa *et al.*, 1970). In view of the fact that this structure was digestible with hyaluronidase, it was considered to represent hyaluronic acid. However, the fact that dermal GAG is composed of nearly an equal volume of HA and dermatan sulphate is well-documented (Noda, 1965; Fleischmajer & Perlish, 1972). Where is dermatan sulphate present in the electron micrograph? We have also been intensely interested in differing features of HA structure as demonstrated with ruthenium red staining (Luft) in the skin of normal controls and the involved skin of lichen myxoedematosus or pretibial myxoedema. Instead of a fine network of ruthenium red-positive filaments as seen in the skin of circumscribed myxoedema, normal skin showed a structure of 'straight threads with knobs' with a tendency to form a loose network. Considering recent works on the proteoglycan macromolecule, we postulated that sulphated GAG, including dermatan sulphate, may be unstainable with routine staining, rather than that the ultrastructure of the GAG has been overlooked (Ishikawa *et al.*, 1976). Thus, in the present study, we have tried to stain the sulphated GAG using a new method. As a result, we have confirmed that HA is linked to probable dermatan sulphate in normal skin, whereas, in the lesion of lichen myxoedematosus, we failed to observe GAG chains bound to HA. Consequently, the circumscribed myxoedema may be characterized not by a simple accumulation of hyaluronic acid, as has been suggested from histochemical and biochemical studies, but by local deposition of HA with no linkages to GAG, although pretibial myxoedema was not re-examined in the present study. The exact mechanism for the appearance of HA unbound to GAG chains in lichen myxoedematosus is still unknown.

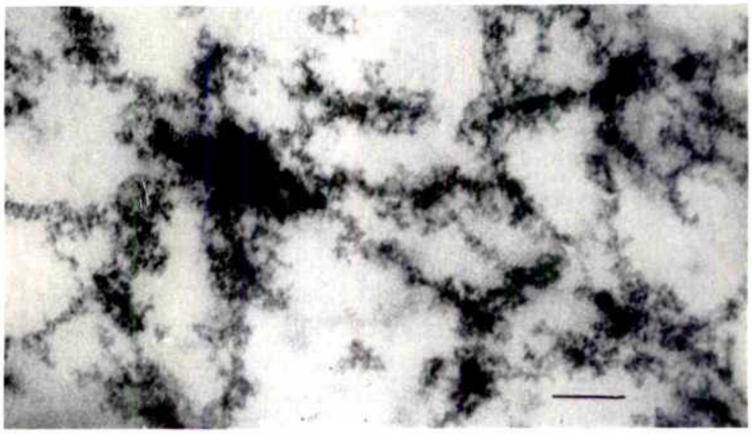

FIGURE 4. Ruthenium red-positive structure seen in bovine articular cartilage (×96,000)

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Epidermal ornithine decarboxylase activity and thymidine incorporation following treatment with ultraviolet A combined with topical 8-methoxypsoralen or anthracene in the hairless mouse

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SUMMARY

Epidermal thymidine incorporation, as a measure of DNA synthesis, and ornithine decarboxylase activity were estimated in hairless albino mice following phototoxic reactions induced by topical anthracene + UV-A, and topical 8-methoxypsoralen (8-MOP) + UV-A. Both treatments caused depression of epidermal thymidine incorporation to 26% of control values at 4 h; this depression persisted through 24 h following 8-MOP + UV-A. Animals treated with anthracene + UV-A showed a fourfold increase in thymidine incorporation at 48 h, declining at 72 and 96 h; after 8-MOP + UV-A increased thymidine incorporation was observed between 4 and 10 days, when a plateau of 96 h duration was observed. After treatment with anthracene + UV-A, epidermal ornithine decarboxylase activity (ODC) was maximal at 4 h, and exhibited a rapid decline, with normal levels at 48 h. Following 8-MOP, UV-A dose-dependent ODC induction occurred: this was later than that induced by anthracene + UV-A with no detectable activity at 4 or 12 h, and maximum activity at 24 h, the elevation persisting through 96 h.

The relationship between ODC induction and epidermal hyperproliferation following these treatments is discussed.

Ornithine decarboxylase (ODC) is the rate-limiting enzyme in polyamine biosynthesis. Increased ODC activity is associated with cell proliferation in a variety of tissues and cell types, including the skin (Janne & Raina, 1968; DiPasquale, White & McGurie, 1978, Mamont *et al.*, 1976; Probst & Krebs, 1975). Stimulation of DNA synthesis, for example by hair plucking (Morrison & Goldsmith, 1978) or ultraviolet-B (UV-B) irradiation (Lowe, Verma & Boutwell, 1978), gives rise to increased levels of ODC activity prior to the increase in DNA synthesis. In psoriatic skin, increased mitotic activity and ODC activity are present (Russell *et al.*, 1978).

Many treatments used in dermatology for proliferative disorders such as psoriasis are aimed at

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reducing epidermal proliferation; corticosteroids, dithranol, psoralens and UV-A and anthracene are believed to act in this way.

In this study, epidermal thymidine incorporation as a measure of proliferation and epidermal ornithine decarboxylase activity have been measured in the hairless mouse following two types of phototoxic reactions, induced by topical 8-MOP and UV-A and topical anthracene+UV-A respectively. These agents were selected as examples of a photodynamic and of a non-photodynamic phototoxic reaction, and because both compounds have been employed clinically in the treatment of psoriasis (J.A.A.D. Editorial, 1979; Walter, 1979). Good correlation between epidermal thymidine incorporation and mitotic counts has previously been demonstrated in this animal model (du Vivier & Stoughton, 1975) using the technique described (Otani, Gange & Walter, 1980) both in skin stimulated by UV irradiation and inhibited by antimitotic agents.

In animal models, experimentally induced depression of DNA synthesis may be followed by a phase of 'rebound' hyperproliferation (Epstein, Fukuyama & Epstein, 1968); the relationship of ODC levels to hyperproliferation induced in this way is of interest.

MATERIALS AND METHODS

Animals

Female albino hairless mice were used for all experiments, between 2 and 4 months of age. Four or five animals were tested in each experimental group.

Light sources

Two UV-A sources were employed. A Blak-Ray lamp (Ultra-Violet Products Inc., San Gabriel, CA 91778) was used for the experiments with anthracene and for some of the 8-MOP studies. This has maximum output in the 360 nm range, with no detectable output at less than 320 nm. Animals were exposed at a distance of 25 cm, providing a dose of 6·7 J/cm^2 over a 45 min period, measured using a Blak-Ray UV-A meter (Model J221).

Four F40 BLB lifeline fluorescent tubes (Sylvania) were mounted 12·5 cm apart in a foil-lined holder; animals were irradiated at a distance of 55 cm. This provided a dose of 3·6 J/cm^2 over a 30 min period, measured using an LM301 UV-A meter (National Biological Corporation). This source was employed for the 10–12 day study of DNA synthesis following 8-MOP.

Anthracene treatment

Anthracene (Sigma) was dissolved by heating in petrolatum at a concentration of 1% (w/v). The solution was mixed on a Vortex mixer as it cooled, to produce a fine suspension. The dorsal skin of each animal was painted with a thin layer of the anthracene preparation using a cotton tip applicator. Excess ointment was removed prior to irradiation, which was performed two hours later. Animals were kept in darkness for 24 h after painting or until sacrifice.

8-*MOP treatment*

The dorsal skin of each animal was painted with 20λ of a preparation of 1% 8-MOP in an alcohol/propylene glycol base (Oxsoralen, Elder).

Irradiation

In most experiments, 8-MOP treated animals received 0·9, 1·8 or 3·6 J/cm^2 of UV-A and anthracene treated animals received 3·0 J/cm^2. These dosages produced similar degrees of suppression of DNA synthesis at 4 h. Where other dosages were employed, this is specified in the text. Animals were irradiated in restraining cages allowing exposure of dorsal skin without significant exposure of ventral skin.

DNA synthesis assay

One hour before sacrifice each animal received 25 μCi ^{3}H-TdR (specific activity 1 Ci/mmol) in 0·2 ml normal saline, by intraperitoneal injection.

TdR incorporation into the epidermis as a measure of epidermal DNA synthesis was estimated by a disc technique (Otani *et al.*, 1980). Animals were sacrificed by neck dislocation. The skin was cleansed with ethanol, excised and spread on a paper towel. Transparent semirigid plastic discs of 17 mm diameter were fixed to the dorsal and ventral skin surfaces with cyano-acrylate glue; excess glue was blotted from the sample. Skins were then heated for 10 min between metal sheets at 60°C to aid the later separation of dermis. The skin surrounding the discs was cut away, and the discs and adherent skin placed in scintillation vials containing 5 ml of 2 M potassium bromide. The samples were then incubated at 60°C for approximately 1 h or until the dermis had contracted and become detached from the epidermis, which remained adherent to the disc. The dermis and potassium bromide were poured from the vial, and the disc and epidermal sample rinsed and incubated in 0·25% acetic acid either for 1 h at room temperature or overnight at 4°C. Each vial was then rinsed with distilled water followed by 95% ethanol and drained, whereupon 5 ml Insta-Gel (Packard) was added to the disc. The ^{3}H content was determined using a Beckman LS-150 scintillation counter.

Ornithine decarboxylase assay

Enzyme activity was determined by measuring the release of $^{14}CO_2$ from DL-(1-^{14}C)-ornithine by the method of Russell & Snyder (1968) with minor modifications.

Enzyme preparation. Animals were sacrificed by neck dislocation; dorsal skin was removed immediately. Epidermis was separated by immersion of the skin in 50 mM phosphate buffer at 56°C for 15 s, followed by immediate cooling in buffer at 0°C; the skin was then spread on paper, epidermis upward. The epidermis was removed by gentle scraping and placed in preweighed tubes surrounded by ice. In each experiment individual skins were assayed; results are the mean of four or five animals. The epidermis was homogenized for 15 s in 19 volumes of 50 mM phosphate buffer containing 0·2 mM pyridoxal phosphate, 1 mM EDTA, and 4 mM dithriothreitol, using a Brinkman homogenizer. The homogenate was centrifuged at 20,000 *g* for 10 min at 0°C, giving a clear supernatant.

Ornithine substrate. 1·2 mM ornithine hydrochloride was dissolved in buffer containing pyridoxal phosphate, EDTA and dithiothreitol as above. 0·5 μCi DL-(1-^{14}C)-ornithine hydrochloride/0·1 ml substrate was added.

Procedure. The enzyme preparation (0·2 ml) was put in each 15 ml tube, 0·1 ml of labelled substrate was added, and the tube closed with a rubber stopper fitted with a central well assembly (Kontes) containing a filter paper bearing 0·05 ml NCS tissue solubilizer (Amersham), for CO_2 absorption. The tubes were incubated for 60 min at 37°C in a shaking water bath. The reaction was terminated by the addition of 0·2 ml 2 M citric acid. Incubation was continued for 1 h to ensure complete absorption of $^{14}CO_2$ by the NCS. Filter papers were then transferred to scintillation vials containing 5 ml of Beta-Max. Each estimation was performed in duplicate; blank tubes contained no enzyme. Release of $^{14}CO_2$ was linear up to 1 h and linearly related to the concentration of epidermal extract.

RESULTS

DNA synthesis

In untreated animals, DNA synthesis, as measured by TdR incorporation, is comparable in dorsal and ventral skin (dorsal/ventral c.p.m. 1 h after ^{3}H-TdR 102% ± 35%, n = 12).

In treated and irradiated animals, suppression of DNA synthesis in dorsal skin to 25% of the ventral (control) value was seen at 4 h, with both anthracene and 8-MOP (Fig. 1a, b). Between 24 and 96 h anthracene plus UV-A (3.0 J/cm^2) treated animals showed increased DNA synthesis in treated skin (Fig. 1a); the increase was maximal at 48 h (419±176% of ventral control value). In contrast, the reduction in DNA synthesis following 8-MOP plus UV-A (0·9 J/cm^2) persisted through 24 h; levels between 48 and 96 h were not significantly different from baseline (Fig. 1b). Treated non-irradiated animals did not show significant alteration in DNA synthesis with the exception of the group sacrificed 96 h after 8-MOP treatment, where a slight (148±33%, $P<0{\cdot}01$) increase was observed. This elevation has not been noted in other experiments.

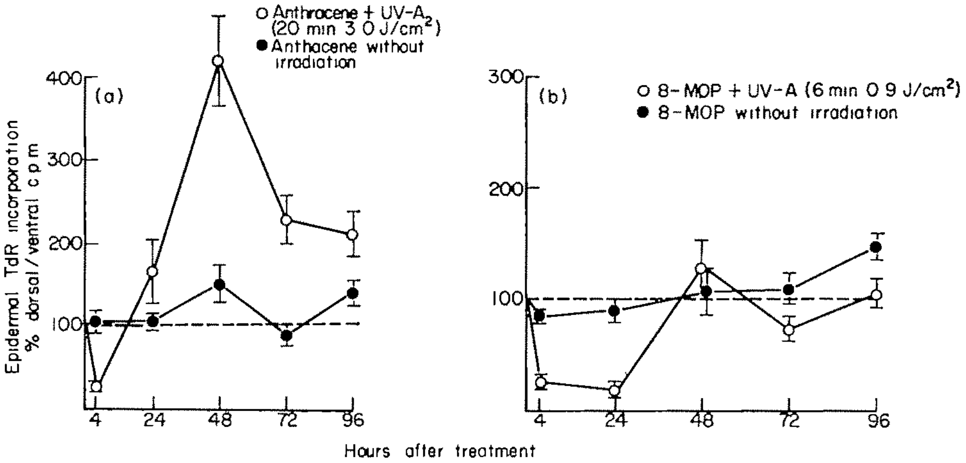

FIGURE 1. Epidermal DNA synthesis, expressed as % TdR incorporation in treated (dorsal) versus control (ventral) skin, at intervals following treatment: (a) anthracene ± UV-A; (b) 8-MOP ± UV-A.

In groups of animals in which the UV-A dosage following 8-MOP was increased fourfold (3·6 J/cm^2) the degree of depression of DNA synthesis wax similar (30% of control value at 4 h) and was of similar duration; again no significant elevation between 48 and 96 h was observed. Irradiation with UV-A alone at dosages of up to 10 J/cm^2 caused no alterations in DNA synthesis.

DNA synthesis was then evaluated over a longer period after treatment with 8-MOP+UV-A. Animals were irradiated with 1·8 J/cm^2 or 3·6 J/cm^2 UV-A using the fluorescent source described, and sacrificed at intervals up to 12 days following treatment (Fig. 2). A similar degree of depression of DNA synthesis at 4 and 24 h was observed. Animals sacrificed at 4, 6 and 8 days after irradiation with 1·8 J/cm^2 UV-A showed significant increase of DNA synthesis (4 and 6 days, $P<0{\cdot}01$; 8 days, $P<0{\cdot}001$) with normal levels at 10 days; after 3·5 J/cm^2 significant elevation was seen at 5, 8 and 10 days (6 days, $P = 0{\cdot}005$; 8 days, $P<0{\cdot}01$; 10 days, $P<0{\cdot}001$).

ODC activity

ODC was not detectable in untreated non-irradiated animals. Irradiation of untreated animals with UV-A at doses of up to 17·9 J/cm^2 did not induce detectable ODC activity at 4 or 24 h in contrast to the effect of UV-B irradiation (Lowe *et al.*, 1978). Animals treated with 1% anthracene without irradiation showed a small but detectable increase in ODC activity at 4 h (0·053±0·01 nmol CO_2/10 mg tissue/h, $P<0{\cdot}005$) compared with untreated controls. Anthracene followed by UV-A irradiation

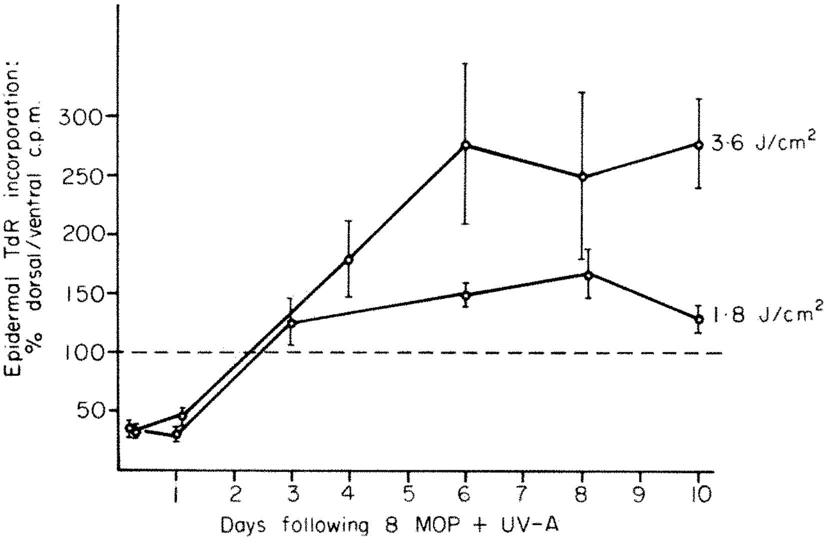

FIGURE 2. Epidermal DNA synthesis, expressed as %TdR incorporation in treated (dorsal) versus control (ventral) skin, at intervals of up to 12 days after 8-MOP+ UV-A (1·8 and 3·6 J/cm²).

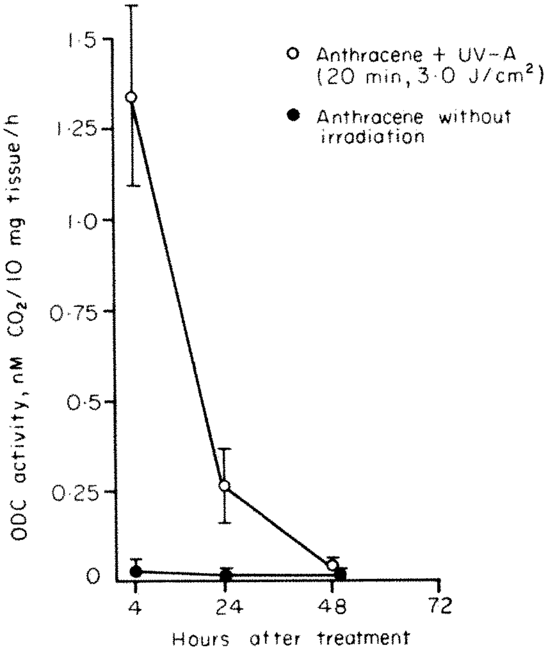

FIGURE 3. Epidermal ODC activity at intervals following treatment with anthracene ± UV-A.

(3·6 J/cm^2) caused a sharp rise in ODC activity (1·32±0·25 nmol CO_2/10 mg tissue/h) (Fig. 3) measured 4 h after irradiation. Elevation persisted through 24 h and declined to almost indetectable levels at 48 h. Animals treated with petrolatum alone, or petrolatum + UV-A (3·6 J/cm^2) did not differ significantly from untreated animals.

In contrast, following 8-MOP + UV-A at a dosage (0·9 J/cm^2) producing a similar degree of suppression of DNA synthesis to that produced by anthracene + UV-A, no induction of ODC activity occurred (Fig. 4). Similarly, no ODC induction was seen with smaller UV-A dosages (0·04 J/cm^2). However, if the dosage of UV-A was increased fourfold (3·6 J/cm^2), high levels of ODC activity were observed. An intermediate degree of elevation was produced by 1·8 J/cm^2. The time course of this ODC induration differed strikingly from that seen following anthracene + UV-A treatment. No significant activity was detectable at 4 or 12 h after irradiation. Elevation was seen at 15 h, with levels maximal at 24 h, elevation persisting through 72 h in the group receiving the greatest amount of irradiation (3·6 J/cm^2). The latter dose represents a many-fold increase in the dose necessary to produce a significant decrease in epidermal thymidine incorporation, which may be seen folllwing as little as 15 s (0.04 J/cm^2) irradiation. A similar degree of 'overdosage' was not feasible following anthracene treatment, in view of the very long irradiation period which would be required. However, some groups of animals were irradiated with 26·8 J/cm^2 and 44·6 J/cm^2 UV-A, representing a 15-fold increase in the dose producing suppression of DNA synthesis to 26% of control values at 4 h. Examined at 24 h, these groups of animals showed similar ODC levels to those seen in animals receiving 3·0

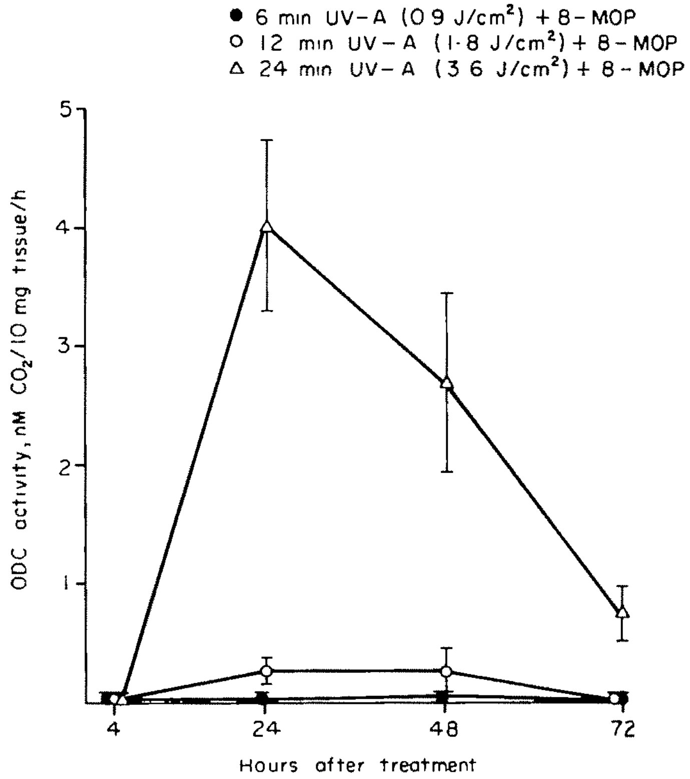

FIGURE 4. Epidermal ODC activity at intervals following treatment with 8-MOP + 0·9 J/cm^2, 1.8 J/cm^2 and 3·6 J/cm^2 UV-A.

J/cm^2 UV-A. The phenomenon of a 'late' increase in ODC following large doses of UV-A in 8-MOP treated animals was thus not demonstrable in anthracene treated animals, nor was the magnitude nearly as great. The degree of 'overdosage' used, however, was somewhat less than that employed in 8-MOP treated animals.

Animals treated with anthracene or 8-MOP showed erythema following irradiation, this was seen at 4 h in anthracene treated animals; in 8-MOP treated animals erythema was not observed until the day following irradiation. 8-MOP treated animals showed marked oedema in the treated areas at UV-A doses of 3·6 J/cm^2. Some scaling was noted from 48 h after treatment in both anthracene and 8-MOP treated animals at doses of 3·0 or 3·6 J/cm^2. Histological signs of inflammation were present at 24 h in 8-MOP treated animals receiving 3·6 J/cm^2 of UV-A, with neutrophil infiltration and oedema of the dermis. Limited patchy damage to the epidermis was also noted, with sunburn cell formation.

These experiments on 8-MOP treated animals have been repeated using the fluorescent UV-A source described and have again shown no ODC induction at 4 or 8 h, with maximum levels of similar magnitude at 24 h after 3·6 J/cm^2 UV-A.

Neither anthracene nor 8-MOP appeared to interfere with the assay of ODC activity, since animals treated with either compound immediately after UV-B irradiation showed no alteration in UV-B induced ODC activity.

DISCUSSION

Both anthracene and 8-MOP required UV-A irradiation to cause suppression of DNA synthesis. Both compounds cause DNA interstrand cross-linking following irradiation (Pathak & Biswak, 1977), and their effect upon DNA synthesis at 6 h has been demonstrated previously (Walter & DeQuoy, 1978).

In the present study, depression of thymidine incorporation was observed with both types of treatment at 4 h. After anthracene + UV-A, this depression was closely followed by a marked 'rebound' with a fourfold increase at 48 h, the elevation persisting through 96 h. Since DNA repair following irradiation occurs early, peaking at 20 min after irradiation in the hairless mouse (Epstein, Fukuyama & Epstein, 1968) and with normal levels of repair at 24 h in human skin (Epstein, Fukuyama & Epstein, 1969), this increase probably represents new DNA synthesis rather than repair.

Following 8-MOP + UV-A, increased levels of thymidine incorporation were not observed before 96 h following irradiation. This is considerably later than the increase seen after anthracene + UV-A, and later than that described after UV-B or UV-C, which is maximum at 48 h (Epstein *et al.*, 1968; Verma, Lowe & Boutwell, 1979). These treatments also cause early induction of ODC: anthracene + UV-A induces maximal activity at 4 h, while UV-B and UV-C induce activity at 4 h which continues to increase through 24 h (Verma *et al.*, 1979). Early ODC induction thus seems to be associated with stimulation of DNA synthesis at 48 h, while the later ODC induction following 8-MOP + UV-A is associated with a later proliferative phase. This is consistent with the hypothesis that polyamine biosynthesis may be important in the pathways leading to stimulation of cell proliferation.

The marked difference in time course of ODC induction after 8-MOP + UV-A suggests that intermediate steps may be involved which are not required for ODC induction by UV-B, UV-C or anthracene + UV-A, or that a different pathway is involved. Other studies also indicate that ODC induction by different agents occurs by different mechanisms. In rat hepatoma cells ODC induction by dibutyryl cyclic AMP and by dexamethasone differs with respect to time course and to inhibition by actinomycin D or polyamines (Canellakis & Theoharides, 1976). ODC induction by hair plucking and by phorbol esters in the rat differs in time course and sensitivity to inhibitors (Lesiewicz & Goldsmith, 1980). Our results also support a distinct pathway for ODC induction by 8-MOP + UV-A, characterized by a latent period of 12 h.

Since ODC normally has a very short half-life of approximately 17 min (O'Brien, Simsiman & Boutwell, 1975), the stimulus for ODC synthesis may be persistent, or the half-life in treated epidermis may be altered. Alterations of half-life have been described in other conditions of stimulation such as hair plucking (Lesiewicz & Goldsmith, 1980).

While the ability of compounds to induce ODC have been proposed as a marker for their tumour promoting ability (O'Brien *et al.*, 1975), recent studies, however, indicate that this relationship is by no means specific or universal (Mufson *et al.*, 1979; Lesiewicz & Goldsmith, 1980), and that the ability to induce ODC may better reflect the degree of epidermal hyperplasia induced. Furthermore it is not possible to extrapolate from chemical tumour promotion to the induction of skin tumours by ultraviolet light. Although UV-B and UV-C are 'carcinogenic principles', ODC induction may be a step in the proliferative response seen following induction rather than a reflection of their carcinogenic potential. It is unreasonable to relate ODC induction in the two chemical phototoxic reactions described to possible carcinogenicity.

Measurements of phototoxicity has in the past largely depended upon measurements of erythema or estimates of histological damage. The observation of specific biochemical changes such as ODC induction occurring in the course of phototoxicity reactions could provide a valuable tool for assessment of phototoxicity and for examining agents with the potential for blocking or inhibiting such reactions.

ACKNOWLEDGMENT

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Effect of hydrocortisone on the adenylate cyclase system of the skin—*in vitro* explant study

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SUMMARY

We have previously reported the effect of hydrocortisone (HC) on the adenylate cyclase system of pig epidermis. HC had no effect on the basal level of cyclic AMP but there was an increased adrenaline-induced cyclic AMP accumulation when epidermal slices were incubated for more than 6 h with HC. This incubation did not alter the responsiveness to histamine. We are interested in the effects on epidermal cells of HC, which may act through the adenylate cyclase system. It is well documented that adrenaline, histamine and adenosine stimulate adenylate cyclase and cause an accumulation of cyclic AMP in pig epidermis. An increased cyclic AMP results in the inhibition of mitosis and epidermal outgrowth. Employing the rate of epidermal outgrowth and mitotic index as indicators of cyclic AMP effect, we studied the effect of HC on the adenylate cyclase system. The pretreatment with HC (100 μM) magnified the inhibitory effect of adrenaline on epidermal outgrowth and mitosis, whereas the inhibitory effect of histamine or adenosine was not affected by HC. It is suggested that HC induced an increased responsiveness of adenylate cyclase to adrenaline, resulting in a magnified inhibitory effect of adrenaline on epidermal outgrowth and mitosis.

Physiological concentrations of glucocorticoids are necessary for a number of metabolic effects mediated by other hormones to occur (Thompson & Lippman, 1974). These so-called 'permissive effects' have been seen in many cellular events including the cyclic AMP dependent process. We have investigated the effect of hydrocortisone (HC) on the adenylate cyclase system of pig epidermis (Iizuka *et al.*, 1980). HC had no effect on the basal level of cyclic AMP. However, there was an increased adrenaline-induced cyclic AMP accumulation when epidermal slices were pre-incubated for more than 6 h with HC. This incubation did not alter the responsiveness to histamine. We are interested in the effects on epidermal cells of HC, which may act through the adenylate cyclase system. Employing an *in vitro* pig explant culture system, we studied the modulatory effect of HC on the adenylate cyclase system of epidermal cells.

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MATERIALS AND METHODS

Skin slices were taken from the back of domestic pigs weighing 4–6 kg, by a Castroviejo keratome set at a depth of 0·2 mm. Squares of side 1–2 mm were cut from the slices and placed dermal side down on glass cover slips which were already placed in plastic Petri dishes. The explants were propagated in RPMI 1640 medium containing penicillin (100 units per ml), streptomycin (100 units per ml) and Fungizone (0·5 μg per ml) supplemented with 10% fetal calf serum. Cultures were maintained at 37°C in a high humidity incubator in a mixture of 5% CO_2 in air. On the 3rd day (unless otherwise stated), outgrowth of epidermal cells was recorded by measurements of the distance from the explant in μm, using a phase contrast microscope with a calibrated scale in the ocular for a pretreatment reading. The rate of growth of these cultures was primarily quantified by measuring the maximum migration of the leading cell nucleus of the epithelial sheet from the original explant. Fresh media containing test drugs were added and the outgrowth distance was determined daily for 3 days. For each concentration in each group of experiments 10–16 explants were run, with the pretreatment readings being between 300 and 600 μm. For histology and mitotic counts, explant cultures were removed from medium, washed three times in cold phosphate buffered saline (PBS), fixed and stained by haematoxylin. Basic procedures are the same as described by Halprin, Leuder & Fusenig (1979).

RPMI 1640 medium was purchased from Gibco (Grand Island, New York). Fetal calf serum was obtained from Microbiological Associates (Walkersville, Md.). Colchicine was the product of Boehringer Mannheim GmbH (Mannheim, W. Germany). Db-cAMP was from P-L Biochemicals, Inc. (Milwaukee, Wis.). Histamine and adenosine were purchased from Wako Chemicals (Osaka, Japan). Antibiotics were purchased from Microbiological Associates (Walkersville, Md.). Propranolol was the product of Sumitomo Chemicals (Osaka, Japan). Hydrocortisone was purchased from Sigma Chemical Co. (St Louis, Mo.). All other chemicals and drugs were of analytical reagent grade.

RESULTS

The effect of cyclic AMP related compounds on outgrowth of pig epidermis in culture (*Table* 1)

The outgrowing distance was measured every 24 h for 3 days following the treatment with or without test drugs. Adrenaline, which induces maximum cyclic AMP accumulation at 50 μM, reduced epidermal outgrowth (43% inhibition). The histamine effect was not dose dependent. Maximum inhibitory effect was found at 100 μM, while 1 mM of histamine was less effective than 100 μM of histamine. Adenosine, which has its maximum effect on cyclic AMP accumulation at 1 mM, inhibited epidermal outgrowth (29% inhibition).

The effect of cyclic AMP related compounds on mitosis of pig epidermis in culture (*Table* 2)

Mitotic index by each test drug added revealed similar results. Percent inhibition of mitotic index by adenylate cyclase stimulators such as adrenaline (50 μM), histamine (100 μM) and adenosine (1 mM) were 40%, 38% and 40%, respectively. Continuous treatment with adrenaline for 3 days in the absence of colchicine also casused mitotic inhibition (46% inhibition at 50 μM of adrenaline). These results indicate that the rate of epidermal outgrowth is comparable with mitotic index in this treatment period.

The effect of HC on epidermal outgrowth in culture (*Fig.* 1)

The outgrowing distance was measured every 24 h following the treatment with or without HC at various concentrations. At 72 h the epidermal outgrowth of 10 μM and 100 μM HC-treated cultures was slightly reduced, but there were no significant alterations in outgrowing distance of HC-treated cultures up to 72 h.

TABLE 1. The effect of cyclic AMP related compounds on outgrowth of pig epidermis in culture

Additions	Outgrowth μM/3 days	% Inhibition
Adrenaline		
0	734·4 ± 43·1 (10)	
5 μM	649·0 ± 40·2 (10)	11·6 n.s.
50 μM	421·9 ± 40·9 (10)	42·6 p<0·01
Histamine		
0	681·3 ± 40·3 (15)	
1 μM	369·2 ± 31·2 (15)	45·8 p<0·01
10 μM	387·3 ± 29·4 (14)	43·2 p<0·01
100 μM	171·4 ± 15·3 (15)	74·8 p<0·01
1 mM	417·5 ± 25·3 (15)	38·7 p<0·01
4-Methyl-histamine		
0	400·0 ± 18·3 (10)	
10 μM	287·5 ± 22·1 (10)	28·1 p<0·01
100 μM	273·5 ± 16·5 (10)	31·6 p<0·01
1 mM	156·3 ± 22·0 (10)	60·9 p<0·01
Adenosine		
0	576·7 ± 35·5 (10)	
100 μM	575·0 ± 29·2 (10)	2·9 n.s.
1 mM	408·8 ± 27·0 (10)	29·1 p<0·01

On the 3rd day after explant of pig skins, epidermal outgrowth was recorded as the pretreatment reading. Culture media were replaced by freshly made RPMI 1640 with 10% fetal calf serum in the presence of test drugs. The outgrowing distance was measured every 24 h for 3 days following the treatment. Each value is the mean of the outgrowth in μM (± s.e.) on the 3rd day after the treatment and expressed as % outgrowth inhibition. Number of explants in brackets. Statistical analysis by paired t-test.

The effect of HC on mitotic index of pig epidermis in culture (*Table* 3)

HC at various concentrations was added to the culture media for an additional 4 h in the presence of colchicine (3·5 μg/ml) following 24 or 48 h treatment with HC. Changes in mitotic index of epidermal cells treated with HC for 28 h were not significant between control cultures and HC-treated cultures. Fifty-two hours treatment with 10 μM or 100 μM HC reduced the mitotic index of epidermal cells.

The effect of adrenaline on epidermal outgrowth following HC treatment

Following 24 h pretreatment with or without HC at various concentrations, these cultures were rinsed with fresh media, then fed by freshly made RPMI 1640 with or without adrenaline (50 μM). The outgrowing distance was measured every 24 h following the treatment. Figure 2 demonstrates that the pretreatment with HC (100 μM) magnified the inhibitory effect of adrenaline on epidermal outgrowth. HC-induced modulations were found as early as 48 h, although the effect of 10 μM HC was not significant during these treatment periods.

The effect of histamine or adenosine on epidermal outgrowth following 24 *h pretreatment with HC*

The same pretreatment with HC as described in Fig. 2 and subsequent treatment with or without histamine and adenosine were performed as shown in Figs 3 and 4. These results showed that HC-pretreatment did not modify the inhibitory effect of either histamine or adenosine on epidermal outgrowth.

TABLE 2. The effect of cyclic AMP related compounds on mitosis of pig epidermis in culture

Additions	Mitotic index ± s.e.	% Inhibition
Adrenaline		
Control	22·0 ± 1 8 (12),* 4 6 ± 0·2 (7)	
5 μM	2·1 ± 0·5 (8)	*55 $P<0\cdot01$
50 μM	13·3 ± 1·4 (10), 2·5 ± 0 4 (9)	40 $P<0\cdot01$, 46 $P<0\cdot01$
Histamine		
Control	51·9 ± 4 1 (19)	
10 μM	37·3 ± 2 9 (19)	28 $P<0\ 01$
100 μM	32·3 ± 2·9 (20)	38 $P<0\ 01$
1 mM	44·6 ± 4 7 (18)	14 n.s.
Adenosine		
Control	41 9 ± 2·4 (13)	
1 mM	25·0 ± 2 4 (13)	40 $P<0\cdot01$

On the 3rd day after explant of pig skins, culture media were replaced by freshly made RPMI 1640 with 10% FCS with test drugs at various concentrations in the presence of colchicine (3·5 μg/ml). After 4 h, explants were rinsed with cold PBS, fixed and stained with haematoxylin. * After 24 h treatment with adrenaline at indicated concentrations, explants were rinsed, fixed and stained. Mitotic index was calculated as mitotic cells per 1,000 cells and expressed as % mitotic inhibition of control. Number of explants in brackets. Statistical analysis by paired t-test.

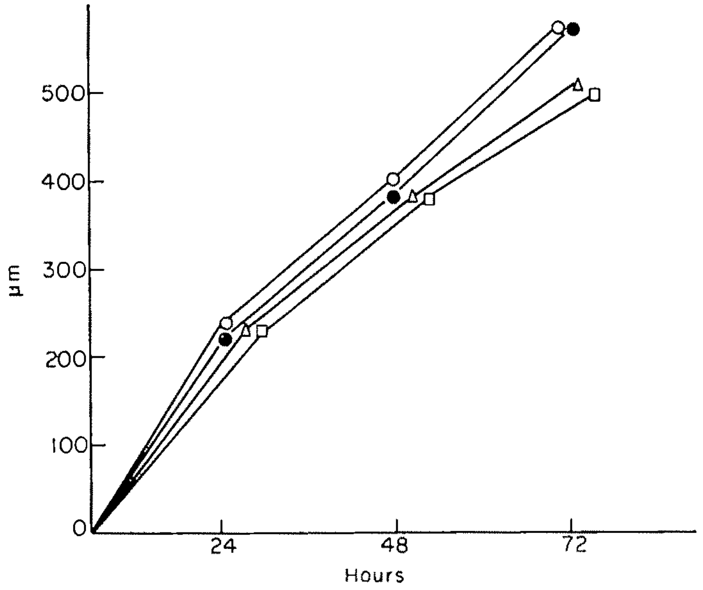

FIGURE 1. The effect of HC on epidermal outgrowth. On the 3rd day after explants of pig skin, culture media were replaced by freshly made RPMI 1640 with 10% fetal calf serum (FCS) with HC at various concentrations. The outgrowth distance was measured every 24 h following the treatment. Each point is the mean of the outgrowth in μm (± s.e.) from sixteen explants. Average s.e. of each point is approximately less than 10%. Control versus each point of HC-treated explant was not statistically significant. Control (○), HC 1 μM (●), HC 10 μM (△) and HC 100 μM (□).

TABLE 3. The effect of HC on mitosis of pig epidermis in culture

	Mitotic index ± s.e.	
Hydrocortisone	24 h	48 h
Control	26·9 ± 2·3 (11)	24·3 ± 1·8 (11)
1 µM	21·8 ± 1·4 (11*)	23·6 ± 3·6 (7*)
10 µM	20·6 ± 1·2 (12*)	19·5 ± 0·4 (7) 0·01 < P < 0·02
100 µM	23·7 ± 2·9 (10*)	18·0 ± 2·2 (7) 0·01 < P < 0·02

On the 3rd day after explant of pig skins, culture media were replaced by freshly made RPMI 1640 with 10% FCS with HC at various concentrations. Following 24 or 48 h treatment with HC, explants were incubated an additional 4 h in the presence of colchicine (3·5 µg/ml) with or without HC. Explants were rinsed with cold PBS, fixed with absolute alcohol and stained with haematoxylin. Mitotic index was calculated as mitotic cells per 1,000 cells (± s.e.). Number of explants in brackets. Statistical analysis by paired t-test.
* Not significant.

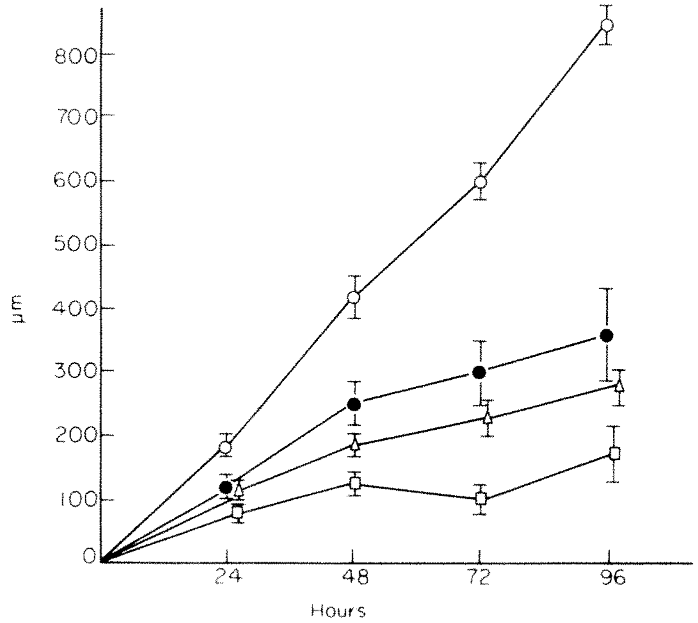

FIGURE 2. The effect of adrenaline on epidermal outgrowth following 24 h pretreatment with HC. On the 3rd day after explant of pig skins, epidermal outgrowth distance was recorded as the pretreatment reading. Culture media were replaced by freshly made RPMI 1640 with 10% FCS with HC at various concentrations. After 24 h pretreatment with HC, the epidermal outgrowth distance was measured and culture media were discarded. After the explants were rinsed with RPMI 1640 media, culture media were replaced by freshly made RPMI 1640 with 10% FCS with adrenaline (50 µM). The outgrowing distance was measured every 24 h following the treatment. Each point is the mean of the outgrowth in microns (± s.e.) from twelve to sixteen explants. At 48, 72 and 96 h, each value pretreated with HC 100 µM was statistically significant ($P < 0{\cdot}01$) compared to each value without HC. Control (○), no pretreatment + adrenaline 50 µM (●), HC 10 µM pretreatment + adrenaline 50 µM (△) and HC 100 µM pretreatment + adrenaline 50 µM (□).

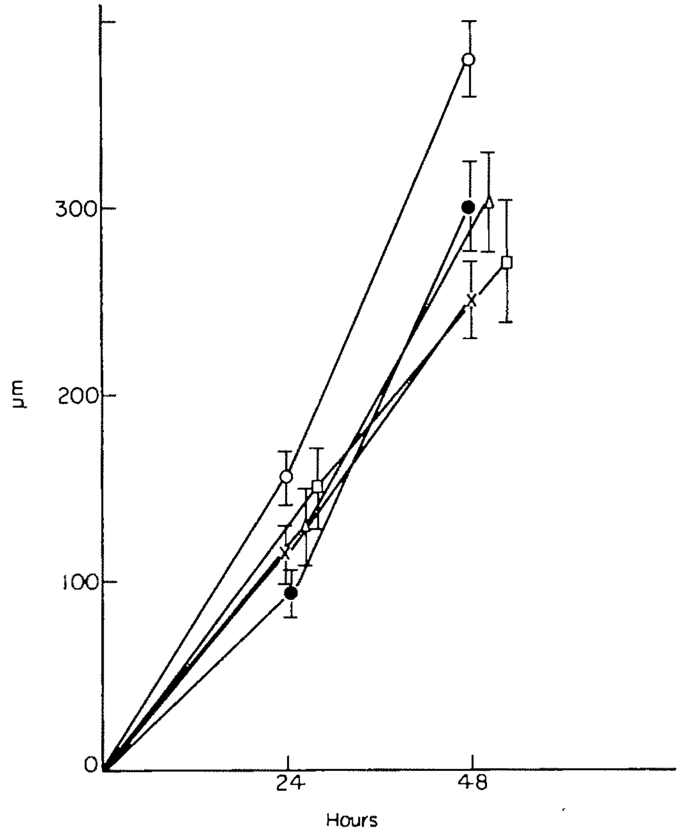

FIGURE 3. The effect of histamine on epidermal outgrowth following 24 h pretreatment with HC. Experimental details are the same as described in Fig. 2 except for the addition of histamine. Each point is the mean of the outgrowth in μM (±s.e.) from twelve to sixteen explants. Not significant between no pretreatment (●) and pretreatment with HC 1 μM (×) or HC 100 μM (□). Control (o), no pretreatment+histamine 100 μM (●), HC 1 μM+histamine 100 μM (×), HC 10 μM+histamine 100 μM (Δ) and HC 100 μM+histamine 100 μM (□).

The effect of adrenaline, histamine and adenosine on mitosis of pig epidermis in culture following 24 h pretreatment with HC

Following 24 h pretreatment with HC at various concentrations, these cultures were rinsed twice with fresh media, then fed by freshly made RPMI 1640 with adrenaline, histamine and adenosine in the presence of colchicine (3·5 μg/ml) for 4 h. Mitoses per 1000 cells were counted in an indicated number of explants. As shown in Table 4, HC-pretreatment magnified the inhibitory effect of adrenaline on mitotic index; it did not modify the inhibitory effect of either histamine or adenosine. These results with mitotic index studies appear to be comparable with the epidermal outgrowth results.

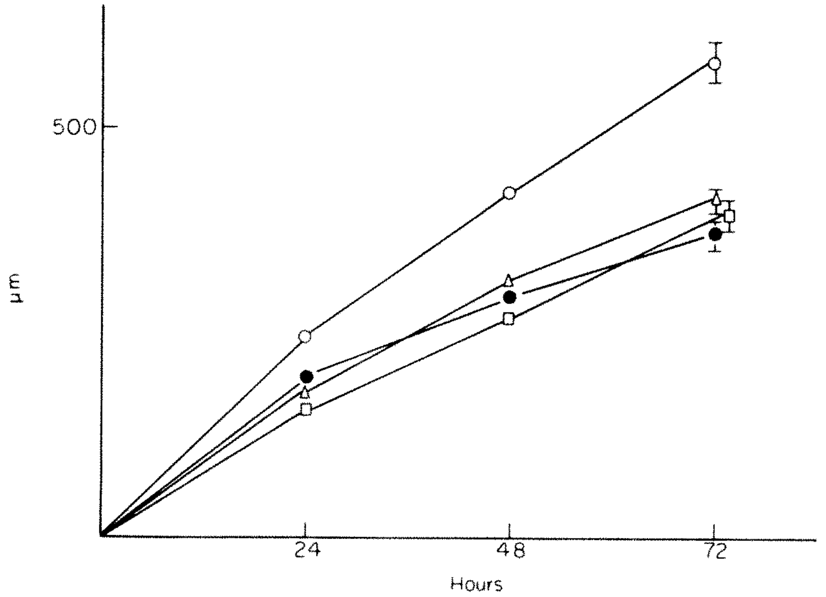

FIGURE 4. The effect of adenosine on epidermal outgrowth following 24 h pretreatment with HC. Experimental details are the same as described in Fig. 2 except for the addition of adenosine. Each point is the mean of the outgrowth in microns (± s.e.) from twelve to sixteen explants. Not significant between no pretreatment (●)and pretreatment with HC 10 μM (△) or HC 100 μM (□). Control (○), no pretreatment + adenosine 1 mM (●), HC 10 μM + adenosine 1 mM (△) and HC 100 μM + adenosine 1 mM (□).

TABLE 4. The effect of adrenaline, histamine and adenosine on mitosis of pig epidermis in culture following 24 h pretreatment, with HC

	Mitotic index mean ± s.e.	% Inhibition	
Adrenaline 50 μM			
0	13·3 ± 1·4 (10)		
HC 10 μM	6·4 ± 0·8 (10)	52	$P<0\cdot01$
HC 100 μM	7·6 ± 0·6 (10)	43	$P<0\cdot01$
Histamine 100 μM			
0	11·1 ± 1·9 (10)		
HC 10 μM	11·5 ± 1·2 (10)	− 4	n.s.
HC 100 μM	12·4 ± 1·6 (10)	−12	n.s.
Adenosine 1 mM			
0	11·3 ± 1·3 (10)		
HC 100 μM	10·2 ± 1·4 (10)	10	n.s.

On the 3rd day after explant of pig skins, explants were pretreated with HC for 24 h. Explants were rinsed by RPMI 1640 and culture media were replaced by freshly made RPMI 1640 with 10% FCS with test drugs and colchicine (3·5 μg/ml). After 4 h incubation, explants were rinsed with cold PBS, fixed and stained with haematoxylin. Mitotic index was calculated as mitotic cells per 1,000 cells ± s.e. and expressed as % mitotic inhibition. Number of explants in brackets. Statistical analysis by paired t-test.

DISCUSSION

Glucocorticoids and cyclic AMP induce similar functions in the epidermis (Voorhees, Marcelo & Duell, 1975). Glucocorticoids inhibit cell proliferation (Fisher & Maibach, 1971; Fisher & Maibach, 1978; Hennings & Elgjo, 1971) and stimulate cell differentiation of the epidermis (Sugimoto & Endo, 1971; Sugimoto *et al.*, 1974). Also these effects can be seen after the treatment of the epidermis with cyclic AMP (Harper, Flaxman & Chopra, 1974a,b; Harper & Flaxman, 1975; Chopra, 1977). Although there are similarities in their functions, it has been postulated that the two compounds work independently. There is little evidence of the interaction of these two compounds in the epidermis. *In vivo* it has been shown that the effect of exercise on mitosis is due to release of adrenaline (Bullough & Laurence, 1961), but Fisher cited that under normal circumstances the diurnal mitotic rhythm is caused by a reciprocal variation in the corticosteroids. On this normal rhythm, adrenaline may superimpose its effect during conditions of stress such as severe exercise (Fisher, 1968).

We reported the effects of hydrocortisone (HC) on the adenylate cyclase system using pig skin slices (Iizuka *et al.*, 1980). Results revealed that HC induced increased responsiveness to adrenaline, resulting in increased accumulation of cyclic AMP in HC-treated skin. Histamine, which is another stimulator of adenylate cyclase of the skin, did not cause any more accumulation of cyclic AMP after this HC-treatment. There was no significant alteration in either low Km or high Km cyclic AMP phosphodiesterase activities by the HC-treatment.

Adrenaline, which induces maximum cyclic AMP accumulation at 50 μM, reduced epidermal outgrowth (43% outgrowth inhibition). The histamine effect was not dose dependent. Maximum inhibitory effect was found at 100 μM, while 1 mM of histamine was less effective than 100 μM of histamine. We have already reported the presence of H_1 and H_2 receptors in pig epidermal cells, and 1 mM histamine stimulated not only H_2-receptors but also H_1-receptors, resulting in increased accumulation of both cyclic AMP and cyclic GMP. On the other hand, 4-methyl-histamine, an H_2-agonist which caused no cyclic GMP accumulation in pig epidermal cells, showed a dose-dependent inhibitory effect of epidermal outgrowth at the concentrations of 1×10^{-5} M—1×10^{-3}M. These dual effects of histamine were discussed previously (Aoyagi *et al.*, 1981a). Adenosine, which had a maximum effect on cyclic AMP accumulation at 1 mM, inhibited epidermal outgrowth (29% outgrowth inhibition). It has been reported (Harper *et al.*, 1974a,b; Harper & Flaxman, 1975; Chopra, 1977) that in an *in vitro* human epidermal system, db-cAMP and related compounds inhibit epidermal proliferation, and probably promote epidermal maturation. Recently Taylor, Halprin & Levine (1980) reported that cyclic AMP and agents which elevate intracellular concentrations of cyclic AMP had no direct effect on the 'S' phase of DNA synthesis. We also reported that db-cAMP inhibited epidermal outgrowth and mitosis of pig epidermal cells in explant culture (Aoyagi *et al.*, 1981b).

Employing these inhibitory effects of adrenaline, histamine and adenosine on epidermal outgrowth and mitosis as parameters of drug effects, we studied the modulatory effect of HC on the adenylate cyclase system. Before examining the modulatory effect of HC, we attempted to determine the adequate concentrations of HC and the adequate incubation time with HC. In our pig epidermal explant system, the rate of epidermal outgrowth started to be slightly inhibited at 72 h by HC (10 μM and 100 μM) but not significantly and mitotic index in the outgrowing epidermis started to be slightly inhibited at 48 h by HC (10 μM and 100 μM). HC did not affect the rate of epidermal outgrowth or mitotic index in the outgrowing epidermis during 24 h treatment.

There are several reports of glucocorticoid effects on epidermal cells. Burke, Kirsch & Charman (1968) stated that cortisone did not alter the rate of epidermal cell turn-over time but reduced epidermal cell mitosis, and inhibited the growth rate of epidermal cells in culture. Gololobola (1970) reported that HC did not inhibit DNA synthesis but delayed the S and G_1 period of epidermal cells.

Fisher (1968) studied the effect of topical corticosteroids on human epidermal mitotic activity and suggested that mitotic inhibition by corticosteroids seemed to be related to their potencies and their concentrations.

We decided to employ 24 h pretreatment with HC for further studies, during which period no inhibitory effect on epidermal outgrowth or mitotic index was recognized. The pretreatment with HC (100 μM) magnified the inhibitory effect of adrenaline on epidermal outgrowth as shown in Fig. 2. HC-induced modulations of the effect of adrenaline were found as early as 48 h, but HC did not alter the effect of either histamine or adenosine for at least 72 h after the treatment. Changes in mitotic index of adrenaline, histamine and adenosine were 40%, 50% and 49%, respectively. Although HC-pretreatment did not alter the inhibitory effect of either histamine or adenosine, it did magnify the inhibitory effect of adrenaline on mitotic index at the concentrations of 10 μM and 100 μM. A rather higher concentration of HC was required to induce the modulations of the adrenaline effect on epidermal outgrowth. Mitotic index seems to be a more sensitive indicator than the rate of epidermal outgrowth as shown in the results of the effect of adrenaline alone on epidermal outgrowth and mitotic index (Tables 1 and 2). These results with mitotic index are comparable to those with epidermal outgrowth.

A possible mode of action of the corticosteroids was suggested by Takeda *et al.*, (1964). These workers demonstrated that the corticosteroids reduced the membrane permeability of dispersed liver cells to certain enzymes and they suggested that this might be the mechanism by which they exert their anti-inflammatory effect. More recently Makman, Nakagawa & White (1967) demonstrated an inhibition of RNA, DNA and protein synthesis in thymocytes exposed to cortisol. They suggested that as a result there would be a decreased formation of membrane transport protein, so the cell membrane would become less permeable to substances requiring this protein. It is quite conceivable that an effect on the membrane would also inhibit mitosis. Our previous data showed that HC worked on the adrenaline-adenylate cyclase system of the skin (Iizuka *et al.*, 1980). Since there was no increased level of cyclic AMP with the addition of HC alone in either short or long period experiments, the effect of HC is not due to the direct activation of adenylate cyclase of the skin. The different adrenaline responses noted in long-term experiments were due mainly to the decreased adrenaline responses in the control skin. HC seems to reveal its action by protecting the adrenaline-adenylate cyclase system which might be more stable in the presence of HC in the media. These observations may be supported by the evidence that when skin slices were incubated with cycloheximide (5 μM) for 48 h, the responsiveness to both adrenaline and histamine was reduced, and the presence of 10% fetal calf serum in the media lessened the decreased adrenaline responses in the control skin (data are not shown). Whatever the mode of HC action may be, our data show that the adrenaline-adenylate cyclase system of the skin can be regulated by HC *in vitro*. As it has been suggested that glucocorticoids and the cyclic AMP system work independently in parallel directions, this effect of HC might be significantly involved in the interaction of these two compounds on the regulatory system of the skin.

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Reduction of increased polyamine levels in psoriatic lesions by retinoid and PUVA treatments

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SUMMARY

Treatment with an oral aromatic retinoid (etretinate) and with PUVA significantly reduced the elevated levels of putrescine, spermidine and spermine in psoriatic lesions. Both treatments also significantly reduced the spermidine/spermine ratio, which is considered to be an indicator of proliferation activity. Although both regimens produced a roughly parallel reduction of epidermal polyamines the initial fall of putrescine was much more rapid in patients receiving retinoid. This may indicate that one of the primary targets of retinoids could be ornithine decarboxylase.

The aliphatic polyamines, putrescine, spermidine and spermine are natural constituents of most living organisms (Jänne, Pösö & Raina, 1978). The diamine putrescine is the precursor of the two polyamines spermidine and spermine. Polyamines are known to participate in several intracellular regulatory processes; their biosynthesis (Fig. 1) and accumulation are intimately associated with growth and differentiation of various tissues (Jänne *et al.*, 1978; Russel & Durie, 1978).

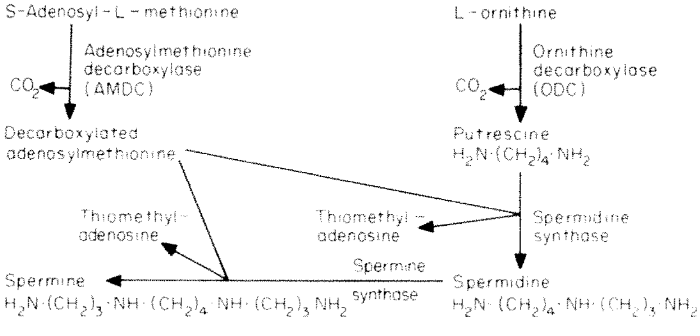
S-Adenosyl-L-methionine
Adenosylmethionine decarboxylase (AMDC)
CO_2
Decarboxylated adenosylmethionine
L-ornithine
Ornithine decarboxylase (ODC)
CO_2
Putrescine $H_2N\cdot(CH_2)_4\cdot NH_2$
Thiomethyl-adenosine
Thiomethyl-adenosine
Spermidine synthase
Spermine
$H_2N\cdot(CH_2)_3\cdot NH\cdot(CH_2)_4\cdot NH\cdot(CH_2)_3\cdot NH_2$
Spermine synthase
Spermidine
$H_2N\cdot(CH_2)_4\cdot NH\cdot(CH_2)_3\cdot NH_2$

FIGURE 1. Polyamine biosynthetic pathway

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Previous studies have revealed that polyamine metabolism is enhanced in psoriasis; the concentrations of putrescine, spermidine and spermine are increased not only in the psoriatic lesions but also in the blood and urine of the patients (Böhlen *et al.*, 1978; Russel *et al.*, 1978; Proctor *et al.*, 1979). Furthermore, the activities of the polyamine synthesizing enzymes L-ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (AMDC) are higher in psoriatic lesions than in normal epidermis (Russel *et al.*, 1978). Treatment with dithranol (Böhlen *et al.*, 1978) with a modified Ingram regimen (Proctor *et al.*, 1979) or PUVA (Grosshans *et al.*, 1980) resulted in a marked fall in the polyamine levels, and topical treatment with a corticosteroid depressed elevated activities of ODC and AMDC in psoriatic lesions (Russel *et al.*, 1978). An aromatic retinoid (etretinate) has been found to be effective in the treatment of psoriasis and disorders of keratinization by a mechanism that so far is not known (Lassus, 1980; Lauharanta, 1980). In animal experiments various retinoids have been able to inhibit chemically induced ODC activity (Verma *et al.*, 1978). The inhibition of ODC has been suggested as a possible mechanism of action of retinoids (Voorhees, 1979; Haddox & Russel, 1979). The aim of our study was to compare the effects of retinoid and PUVA treatments on polyamine levels in psoriatic lesions.

MATERIALS AND METHODS

Patients and treatments

Eight psoriatic patients (mean age 52.4 years; range 31–72 years) were treated with an aromatic retinoid (etretinate, Tigason), and seven patients (mean age 43.4 years; range 27–68 years) with PUVA. Tigason was administered at an initial daily dose of 0·8–0·9 mg/kg which later was gradually reduced to 0·4–0·5 mg/kg depending on the response. PUVA treatment was given three times per week. The 8-methoxypsoralen was administered at a dose of approximately 0·6 mg/kg body weight, followed 2 h later by irradiation in a high intensity cabin (PUVA-22, Airam Ltd, Helsinki, Finland). Control specimens were taken from ten healthy persons (mean age 33·6 years; range 28–42 years).

Collection of samples

The 4 mm whole skin punch biopsy specimens from psoriatic lesions were taken before treatment and after 1, 2 and 4 weeks of treatment; the remission sample was taken from the site of the previous plaque. Samples were frozen in liquid nitrogen and stored at −50°C until processed.

Determination of polyamines

For polyamine determinations the skin samples were homogenized in 0·2 N perchloric acid using an all-glass Potter-Elvehjelm tissue homogenizer. Polyamines were determined as their fluorescent DANS derivatives by the method of Seiler (1970), with modifications introduced by Dreyfuss *et al.* (1973). Putrescine, spermidine and spermine, as hydrochloride salts, used as standards were obtained from Calbiochem (San Diego, California).

Statistical analyses

Significances were tested using the Student's t-test.

RESULTS

Remission was achieved in six of the eight patients treated with retinoid, and in all the seven patients treated with PUVA.

TABLE 1. Levels of putrescine, spermidine and spermine (nmol/g tissue) and the spermidine/spermine (Spd/Sp) ratio during retinoid treatment. Each figure represents the mean ± s.e. mean

Time	n	Putrescine	Spermidine	Spermine	Spd/sp
0	8	75 ± 14	444 ± 68	300 ± 48	1·48 ± 0·04
1 week	8	41 ± 5	350 ± 31	278 ± 47	1·46 ± 0·21
2 weeks	7	44 ± 4	338 ± 23	263 ± 43	1·43 ± 0·27
4 weeks	8	35 ± 6	278 ± 36	235 ± 32	1·23 ± 0·11
Remission	6	21 ± 3	151 ± 23	172 ± 21	0·87 ± 0·05
Healthy controls	10	29 ± 2	100 ± 10	132 ± 13	0·77 ± 0·04
Significance					
Pretreatment versus control levels		$P<0{\cdot}01$	$P<0{\cdot}001$	$P<0{\cdot}01$	$P<0{\cdot}001$
Pretreatment versus remission levels		$P<0{\cdot}05$	$P<0{\cdot}01$	$P<0{\cdot}05$	$P<0{\cdot}001$

The mean treatment period with retinoid was 9·7 weeks (range 6–16 weeks) and with PUVA 7·7 weeks (range 6–11 weeks).

Table 1 presents the changes in polyamine levels during retinoid treatment. During the first week there was a rapid decrease (45%) in the mean putrescine concentration ($P<0{\cdot}01$). Finally putrescine fell even below the level of the ten healthy controls. The decrease in the mean spermidine concentration after 1 week was about 20% but was statistically not significant ($P<0{\cdot}1$). In the spermine level there occurred only a slight response during the first week. At remission the concentrations of spermidine and spermine remained at somewhat higher levels than those of the controls but the difference was statistically not significant (spermidine $P<0{\cdot}1$; spermine $P<0{\cdot}5$). The decrease was more pronounced for spermidine than for spermine, resulting in a highly significant ($P<0{\cdot}001$) reduction in the spermidine/spermine ratio, which finally fell almost to the level of the healthy controls.

Table 2 gives the polyamine levels during PUVA treatment. After the first week only a minor response was seen. After 2 weeks, however, a definite decrease started towards normal values. At remission the concentrations of spermidine and spermine remained somewhat above control values, as also was the case with retinoid treatment. The difference in the spermine level between the PUVA treated group at remission and the controls was almost significant ($P<0{\cdot}05$). The corresponding difference in the spermine level was not significant ($P<0{\cdot}5$).

TABLE 2. Levels of putrescine, spermidine and spermidine (nmol/g tissue) and the spermidine/spermine (Spd/Sp) ratio during PUVA treatment. Each figure represents the mean ± s.e.mean

Time	n	Putrescine	Spermidine	Spermine	Spd/Sp
0	7	64 ± 10	423 ± 93	320 ± 68	1·30 ± 0·06
1 week	7	61 ± 9	405 ± 77	304 ± 52	1·32 ± 0·07
2 weeks	6	47 ± 13	303 ± 47	240 ± 27	1·23 ± 0·07
4 weeks	6	36 ± 5	219 ± 45	186 ± 40	1·18 ± 0·07
Remission	7	27 ± 5	149 ± 20	147 ± 20	1·02 ± 0·06
Healthy controls	10	29 ± 2	100 ± 10	132 ± 13	0·77 ± 0·04
Significance					
Pretreatment versus control levels		$P<0{\cdot}01$	$P<0{\cdot}001$	$P<0{\cdot}02$	$P<0{\cdot}001$
Pretreatment versus remission levels		$P<0{\cdot}02$	$P<0{\cdot}02$	$P<0{\cdot}05$	$P<0{\cdot}01$

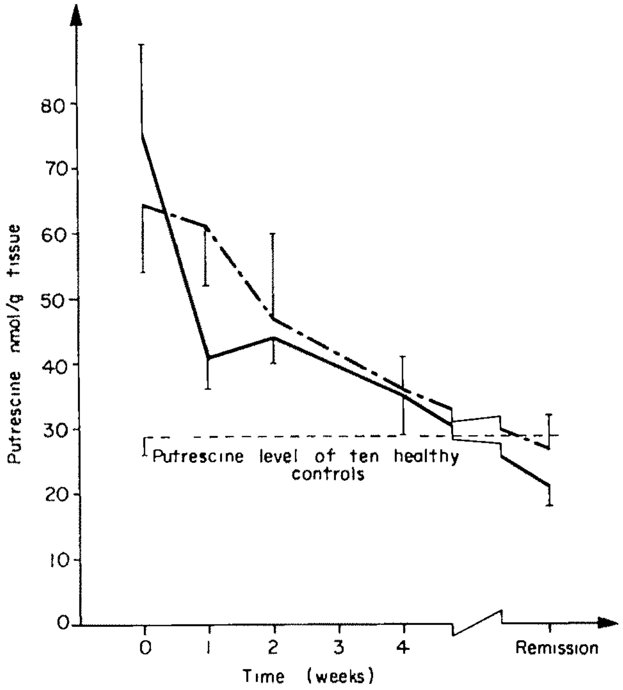

FIGURE 2. Changes in putrescine levels (mean ± s.e.mean), during retinoid (——) and PUVA (–·–·–) treatment.

Figure 2 shows the fall of putrescine during retinoid and PUVA treatments. The response of putrescine to the retinoid treatment was much more rapid than that to PUVA after the first week ($P < 0\cdot05$).

DISCUSSION

The results of the present study show that both retinoid and PUVA treatments have a normalizing, or at least a nearly normalizing, effect on the significantly elevated levels of putrescine, spermidine and spermine in psoriatic lesions. The initial levels of polyamines in our study are in agreement with those of Böhlen *et al.* (1978) who also used whole skin biopsies and expressed the concentrations as nmol/g tissue as we have done. In our study the mean levels of putrescine, spermidine and spermine were decreased by 72%, 66% and 43%, respectively with retinoid treatment and by 58%, 65% and 54% with PUVA treatment. These decreases were more pronounced than those reported by Proctor *et al.*, (1979) who achieved 50% (putrescine), 24% (spermidine) and 35% (spermine) reductions with a modified Ingram regimen. They have expressed the amount of polyamines as pmol/μg of DNA in contrast to our nmol/g tissue, which, to some extent, may explain the difference. Both our treatments resulted in a significant decrease in the spermidine/spermine ratio. The study of Grosshans *et al.* (1980) shows a similar trend, contrary to Proctor *et al.*, (1979) who observed an elevated ratio after treatment. The molar spermidine/spermine ratio is considered to be an indicator of proliferative activity; it is high (even $>2\cdot0$) in tissues undergoing hypertrophy and/or hyperplasia, and low ($<1\cdot0$) in tissues with low proliferative activity or in differentiated tissues with a slow rate of RNA and protein synthesis (Jänne *et al.*, 1978; Russel & Durie, 1978). A decreased spermidine/spermine ratio could therefore be expected as a result of successful antipsoriatic treatment.

The first sign of improvement during retinoid treatment is the loss of psoriatic scale, often seen during the first week of treatment. Reduction of infiltration usually becomes evident later. Dierlich *et al.* (1979) found that oral retinoid has a distinct normalizing effect on proliferation but with a latency of three weeks. Light and electronmicroscopic studies of Orfanos & Runne (1978) have revealed that the first signs of improvement of disturbed keratinization in psoriasis appear after about 2 weeks of retinoid treatment. In our study the retinoid treatment resulted in a marked decrease in the putrescine level already during the first week. In the polyamine biosynthesis, putrescine is formed from ornithine in a reaction catalyzed by ornithine decarboxylase (ODC). There are several observations of the close correlation between ODC activity and the putrescine level (McCann *et al.*, 1975; Jänne *et al.*, 1978; Mamont *et al.*, 1978; Verma *et al.*, 1978; Seiler & Knödgen, 1979). Consequently, the levels of putrescine closely reflect the ambient ODC activity. After 2 weeks treatment of psoriasis with difluoromethylornithine (an irreversible inhibitor of ODC), Grosshans *et al.* (1980) found a marked decrease in putrescine and spermidine but not in spermine. In our study, retinoid treatment had quite a similar effect after 1 week. From the above observations it is obvious that the effect of retinoid on polyamine metabolism precedes its effects on hyperproliferation and disturbed keratinization in psoriatic lesions. Our findings are compatible with the theory that the therapeutic action of retinoids could be mediated by this mechanism. Furthermore, our preliminary results point to a reduction in ODC activity during retinoid treatment.

In an autoradiographic study Hofmann *et al.* (1980) found that there is a pronounced decrease in labelled cells within the first week of PUVA treatment. The normalizing effect of PUVA treatment on polyamine metabolism becomes evident somewhat later, suggesting that the observed changes may be secondary to the obvious inhibitory effects of PUVA on DNA replication. The differences in the initial autoradiographic and polyamine responses during retinoid and PUVA treatments indicate that the intracellular mechanisms involved in the antipsoriatic action of these modalities are not the same. The concerted action of these mechanisms may explain the synergistic effect of the combination treatment with retinoid and PUVA (RePUVA) (Lauharanta, Juvakoski & Lassus, 1981).

A recent finding of stimulation of DNA synthesis in normal mouse skin by topical putrescine (Gange & Dequoy, 1980) gives further support to the supposition that polyamines may participate in the regulation of epidermal proliferation. However, the role of polyamine metabolism in the pathophysiology of psoriasis cannot as yet be fully defined. The accumulation of polyamines in psoriasis and their normalization during treatment suggest that the pathological processes involve polyamines. Therefore, the use of inhibitors of polyamine biosynthesis may have practical application in the treatment of proliferative disorders of the skin.

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The selective culture of keratinocytes using a cytotoxic antifibroblast monoclonal antibody

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SUMMARY

A technique is described for the selective killing of fibroblasts in primary cultures and subcultures of human keratinocytes using a monoclonal antibody raised against human fibroblasts. The antibody kills fibroblasts by complement mediated cytotoxicity but is not toxic to keratinocytes.

The last few years have seen an increasing awareness among many experimental biologists that work on animal fibroblast cell lines has only limited application to the study of both normal and malignant human tissues and many workers are developing techniques for the serial culture of human epithelial cells (Stoner *et al.*, 1978) and in particular keratinocytes derived from both skin (Rheinwald & Green 1975) and mucous membranes (Gusterson & Monaghan, 1979; Taichman, Reilly & Garant, 1979). Although it is possible to obtain relatively pure populations of epithelial cells using selective culture conditions (Rheinwald & Green, 1977), contamination with fibroblasts is still a problem to many workers using primary cultures. We report the routine use of a cytotoxic monoclonal antibody raised against human fibroblasts for the primary culture of keratinocyte preparations derived from human buccal mucosa. The antibody does not appear to be toxic to keratinocytes and provides a simple method, hitherto not available, for the purification of keratinocytes for both primary and serial culture.

MATERIALS AND METHODS

Culture of keratinocytes

Buccal mucosa removed from patients undergoing minor oral surgery for impacted wisdom teeth was transported to the laboratory in 199 Culture Medium. The tissue was used within 3 h of surgery. Explant cultures were set up as previously described (Gusterson & Monaghan, 1979). Cultures 21 days old were treated with 0·025% trypsin and the cells subcultured to 25 cm^2 flasks at a density of 5×10^{-5} cells/cm^2. Cells replated at this density attached and continued to proliferate.

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Use of the monoclonal antibody

The production of the antibody used in these experiments has been previously described (Edwards, Foster & McIlhinney, 1980). The antibody (LICR LON/FIB86) belongs to subclass γ2b and was raised to fibroblasts derived from normal human breast using the hybrid myeloma or 'hybridoma' system (Milstein *et al.*, 1979). The antibody was prepared in the form of ascitic fluid and stored prior to use at -40°C. The source of complement was rabbit serum which was stored in aliquots in liquid nitrogen.

The antibody was used routinely to remove fibroblasts from 21 day old primary cultures prior to subculture and from subcultures 48 h after replating. Both methods have proved effective although using the re-seeded populations of cells facilitated access of the antibody to fibroblasts trapped beneath the keratinocytes in primary cultures. The protocol used was the same in both cases.

The cultures were washed twice in serum-free Dulbecco's Modified Eagle's medium. The antibody at a final dilution of 1 in 400 in 199 Culture Medium, using 5 ml per 25 cm^2 flask, was incubated with the cells at room temperature for 15 min. The antibody was decanted from the cells which were washed three times with serum-free medium to remove any free antibody. Whole rabbit serum, as a source of complement, was diluted in 199 Culture Medium and used at a dilution of 1 in 20 with a total volume of 3 ml per 25 cm^2 flask. Incubation with the complement was for 45 min at 37°C. The cultures were washed three times with serum-free medium and returned to their normal culture conditions (Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum).

Control cultures were treated with antibody or the complement alone using the same protocol.

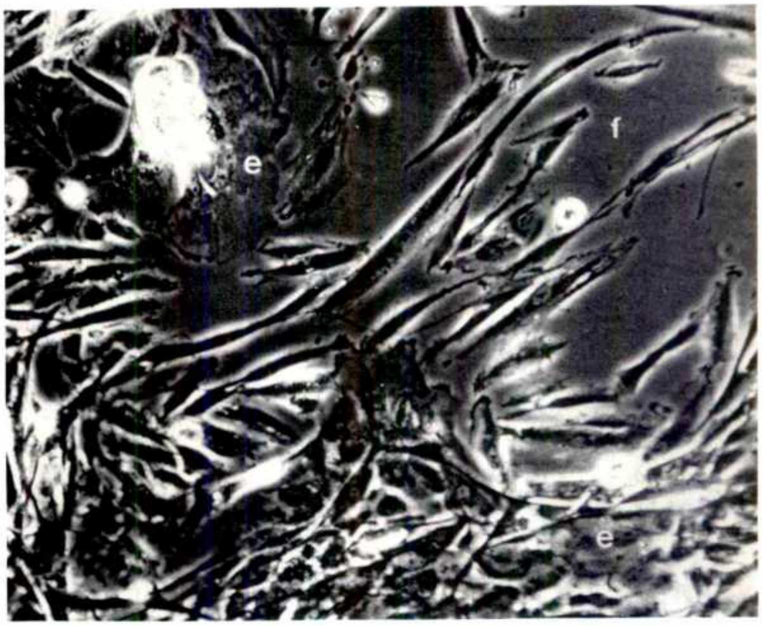

FIGURE 1. Phase contrast photomicrograph of a 21-day culture of human buccal mucosa demonstrating epithelial cells (e) and a focus of fibroblasts (f) (× 12).

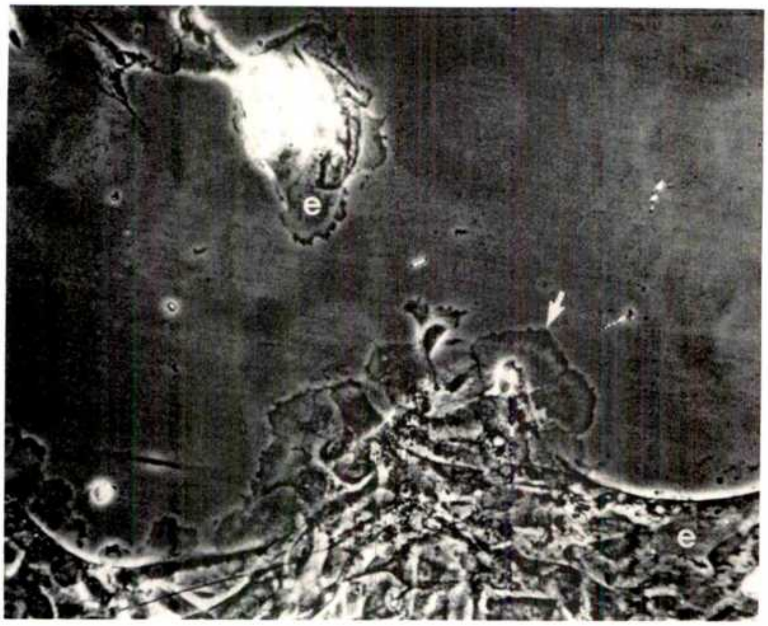

FIGURE 2. Phase contrast photomicrograph of the same field as Fig. 1 after treatment with antifibroblast monoclonal antibody. Note epithelial islands (e) and absence of fibroblasts. Ruffled membranes of epithelial cells (arrow) indicate lack of toxic effect to epithelial cells (× 12).

RESULTS

The cells present in primary cultures of oral epithelium have been previously described (Gusterson & Monaghan, 1979). The explants attach within 24 h and after 48–72 h keratinocytes start to migrate out from the explant to form a sheet of rapidly dividing cells. These migrating cells which have been shown to be keratinocytes form an organized three dimensional structure closely approximating to that seen *in vivo* with differentiation in culture to produce the typical cornified envelope of the mature keratinocyte (Gusterson & Monaghan, 1979). After 3 weeks, however, occasional fibroblasts are seen in islands between the sheets of epithelial cells (Fig. 1). On subculture the fibroblasts proliferate very rapidly and become the predominant cell type. As can be seen in Fig. 2, the fibroblasts are removed by the antibody leaving clean keratinocyte cultures. Figure 3 demonstrates the same culture 48 h after treatment; the presence of numerous mitotic figures in the epithelial cells indicates that the antibody is not cytotoxic to the keratinocytes. The cultures treated with complement and antibody alone showed no toxicity to either the fibroblasts or to the epithelial cells.

It is our experience as with other groups that it is not possible to subculture keratinocytes at low density without the use of feeder layers or other culture manipulations (Rheinwald & Green, 1975; Taichman *et al.*, 1979). In many instances, however, it is necessary to have purified epithelial cells without contamination by feeder cells or other matrices. We have found that by seeding at high density limited re-passaging is possible, and the use of the antifibroblast monoclonal antibody 48 h after subculture removes those fibroblasts trapped in the outgrowth in the primary cultures and previously inaccessible to the antibody.

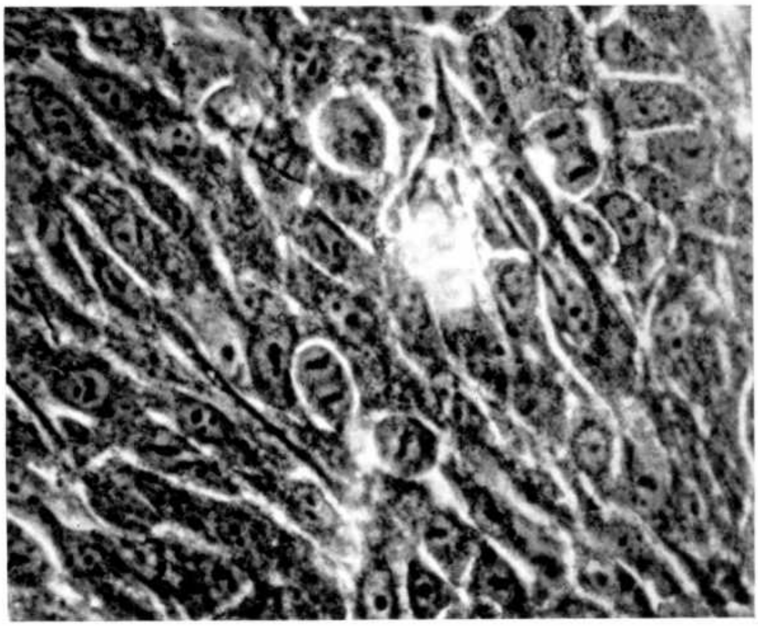

FIGURE 3. Phase contrast photomicrograph of epithelial cells 48 h after treatment with antifibroblast antibody with numerous mitoses and epithelial pavement morphology (× 48).

DISCUSSION

Two major problems have deterred many workers from using human epithelium for *in vitro* studies. The first was the difficulty in establishing such cultures and until recently it has not been possible to serially passage human keratinocytes (Rheinwald & Green, 1975). The other major problem has been overgrowth by fibroblasts and although many techniques have been suggested for the selective growth of epithelial cells (Duskin, Maoz & Fuchs, 1975; Webber & Chaproniere-Rickenberg, 1980; Kao & Prockop, 1977; Fritsch, Tappeiner & Huspek, 1979) they are all rather capricious, and resort to manual scraping is still the time honoured answer to this problem of most tissue culturists. The method described provides an answer which in our hands has removed the tedium of scraping or the biochemically awkward alternative of using feeder layers as a means of controlling fibroblasts.

The method described here provides the opportunity of producing epithelial enriched cultures from tissues derived from a number of sources. We are currently developing a technique for the large scale production of keratinocytes from mammoplasties using a combination of the techniques described here and those reported by Rheinwald & Green (1975) for the continuous passage of keratinocytes. Such developments will provide large quantities of cells for both biochemical and morphological studies. Provisional results also suggest that the antibody may be a useful adjunct in the establishment of human tumour cell lines (Edwards, Easty & Foster, 1980). It can be concluded that this technique will be a useful tool in the expanding field of *in vitro* studies on normal and malignant keratinocyte populations.

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Suppression of active Arthus reaction by colchicine

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SUMMARY

An effect of colchicine on the active Arthus reaction in rabbits was investigated *in vivo* with simultaneous evaluation of *in vitro* chemotactic activity of polymorphonuclear leukocytes. Arthus reactions were induced by intracutaneous injection of ovalbumin into rabbits preimmunized with ovalbumin. Colchicine, 1 mg/kg or 2 mg/kg, injected intraperitoneally one hour prior to the induction of the Arthus reaction, suppressed erythema and induration at 12 h, when histology showed decreased numbers of infiltrated polymorphonuclear leukocytes and no apparent vascular damage. Perivascular deposits of C3, suggestive of the presence of immune complexes, persisted to 24 h. *In vitro* chemotaxis of polymorphonuclear leukocytes using Boyden chamber techniques revealed suppression of the activity in the colchicine-treated animals at 6 and 12 h.

These findings suggest that colchicine inhibits vascular injury by interfering with directional chemotaxis of polymorphonuclear leukocytes to the lesional sites.

Colchicine has been used successfully for the treatment of cutaneous vascular diseases, such as necrotizing vasculitis (Hazen & Michel, 1979) and Behçet's disease (Miyachi *et al.*, 1981), in which polymorphonuclear leukocytes (PMNs) play an important role. Necrotizing vasculitis perhaps represents an Arthus reaction in which vessel wall necrosis is induced by PMN infiltration. Current evidence strongly suggests that these cells respond to chemotactic factors released by immune complex deposits in the vessel wall (Sams, 1980). Behçet's disease is also considered to be an Arthus-like reaction (Cooper, Penny & Fiddes, 1971), although its pathogenesis is not established.

To investigate how colchicine works in these diseases, we studied the *in vivo* effects of colchicine on the active Arthus reaction, a model of immune complex injury.

MATERIALS AND METHODS

Active Arthus reaction

Antibody against ovalbumin was raised in six albino rabbits weighing 2·5–3 kg by four intravenous injections of 2 mg of ovalbumin in saline. Eight days after the last immunization, when the presence of the antibody was confirmed on immunodiffusion plates, active Arthus reactions were induced in two sites of the back skin by injecting intracutaneously 0·2 ml of 2·5% ovalbumin solution.

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Colchicine treatment

Colchicine (Sigma) was freshly diluted with saline at a concentration of 0·25 mg/ml. One hour before the induction of active Arthus reactions, animals were administered a single intraperitoneal injection of this solution to give a final dose of 1 mg/kg or 2 mg/kg. Controls were given saline intraperitoneally.

Gross and microscopic examinations

Gross skin changes were evaluated 12 and 24 h after the injection of ovalbumin, and biopsy specimens were taken at the same time. A half of each tissue was fixed in 10% formalin for haematoxylin and eosin stain to score the grading of infiltrated PMNs. The other half was quick-frozen and 6 μm cryostat sections were prepared. They were stained with FITC-labelled anti-rabbit C3 antisera (1:20 diluted, F/P ratio:2.30 mg/g, Cappel Lab.) for 30 min at 37°C.

In vitro chemotactic assay

PMN chemotactic activity was determined using modified Boyden chambers. Casein sodium (5 mg/ml) was used as a chemotactic factor. Blood PMNs from the animals at 6, 12 or 24 h were suspended in Hanks balanced salt solution at a concentration of 2×10^6/ml. The cell suspensions were placed in the upper compartment of the chambers and were incubated in a humidified atmosphere with 5% CO_2 at 37°C for 3 h. Millipore filters were fixed in methanol, stained with haematoxylin and mounted on a glass slide with the test chamber-side uppermost. For each filter, 10 random fields were counted at a magnification of ×400 and the number of PMNs migrating completely through to the bottom surface of the filter was recorded. Chemotactic activity was expressed as the average number of cells per high power field.

White blood cell (WBC) count

Total counts and per cent of PMNs in peripheral leukocytes were examined before and 24 h after colchicine treatment.

RESULTS

The results are summarized in Table 1.

TABLE 1. Scoring of degrees of gross and microscopic observations*

Animals group	Hours after induction of Arthus reaction	Gross observations			Histological findings		IF findings: perivascular C_3 deposition
		Induration	Erythema	Haemorrhage	PMN infiltration	RBC extravasation	
Non-treated	12	+	+	+	+	+	+
(1)	24	+	++	++	++	++	−
Colchicine treated							
(1 mg/kg)	12	−	−	−	±	+	+
(2)	24	+	+	+	+	+	+
Colchicine treated							
(2 mg/kg)	12	−	−	−	−	+	+
(3)	24	−	+	+	+	+	+

* Scored into 4 degrees: (−) negligible, (±) slight, (+) moderate, (++) marked.

Gross skin changes

By 12 h, erythema and induration with central haemorrhage occurred in control animals, while neither of these changes was visible in the colchicine-treated ones (1 mg/kg and 2 mg/kg). At 24 h, the intensity of haemorrhage in colchicine-injected animals was as pronounced as in the controls. Induration was detected in animals injected with 1 mg/kg of colchicine but not with 2 mg/kg of colchicine.

Histology

At 12 h, a dermal infiltrate composed mainly of PMNs and extravasated red blood cells (RBCs) was noted both in treated and in control animals, though PMN infiltration was always more massive in the control group. At 24 h, the number of infiltrated PMNs was markedly reduced by colchicine, but the severity of haemorrhage was unchanged (Fig. 1). In colchicine-treated animals, blood vessel walls were not apparently damaged as compared with controls.

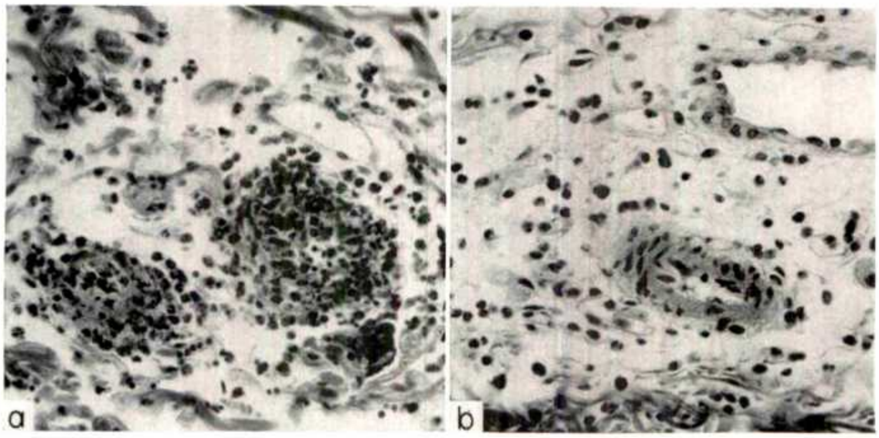

FIGURE 1. Microscopic changes in Arthus reaction at 24 h (a) in control and (b) in colchicine treated animals.

Immunofluorescence (IF) study

A granular deposition of C_3 was detected perivascularly in the mid-dermis at 12 h and even at 24 h in the colchicine-treated animals, whereas in the control animals, immunofluorescence showed granular deposits of C3 around the vessel walls at 12 h which were cleared away by 24 h (Fig. 2). Control sites of immunized animals revealed no specific fluorescence.

In vitro chemotactic activity

The effect of colchicine on directional locomotion and random movement is shown in Table 2. Directional locomotion of PMNs induced by casein sodium was apparently affected by colchicine at 6 and 12 h. However, this effect was no longer observed at 24 h. In contrast, random movement was not influenced by colchicine treatment.

WBC count

The number of peripheral leukocytes and the percent of PMNs were comparable before and 24 h after the injection of colchicine.

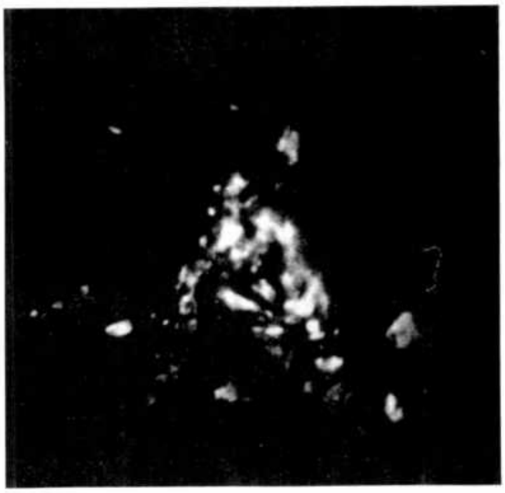

FIGURE 2. Granular deposition of C_3 around vessel wall at 24 h in colchicine-treated animal (2 mg/kg).

TABLE 2. The effect of colchicine on directional locomotion and on random movement

		PMNs/10 high power fields* Time (h)		
		6	12	24
Directional movement	Colchicine treated (2 mg/kg)	119·0 ± 8·39†	139·0 ± 16·64†	369·0 ± 38·16‡
	Control	439·67 ± 36·29	361·67 ± 15·17	489·33 ± 13·53
Random movement	Colchicine treated (2 mg/kg)	39·33 ± 7·69§	47·0 ± 7·0§	33·0 ± 9·86§
	Control	42·33 ± 8·09	53·33 ± 2·73	36·0 ± 4·73

* Mean ± s.e.; † $P < 0{\cdot}01$; ‡ $P > 0{\cdot}05$; § $P > 0{\cdot}1$.

DISCUSSION

In control rabbits, erythema and haemorrhage reached a maximum at 24 h, when PMN infiltration was most intense. On the other hand, C3 deposition, suggestive of the presence of immune complexes, observed at 12 h, was not detectable at 24 h. This has been explained by ingestion and subsequent degradation of immune complexes by infiltrating PMNs. Colchicine-treated animals showed neither induration, erythema nor haemorrhage at 12 h. Histologically, infiltration by PMNs was much less intense in a group treated with 1 mg/kg of colchicine and negligible with 2 mg/kg of colchicine compared with the control group. Although there was microscopic haemorrhage around vessels gross examination could not detect this.

In vitro chemotactic assay revealed that PMN chemotaxis in colchicine-treated animals was suppressed at 6 and 12 h but not at 24 h on *in vitro* Boyden chamber examination. At 24 h, weak erythema and haemorrhage occurred in both the 1 mg/kg and 2 mg/kg colchicine-treated group and, at the same time, PMNs were infiltrating and RBCs extravasated. C_3 was deposited perivascularly by 12 and 24 h. Thus, persisting deposition of C_3 in colchicine-treated groups might result from decreased infiltration of PMNs at the lesion. It is of interest that extravasation of RBCs was observed around the vessel with deposits of C_3 in the absence of PMN infiltration at 12 h in group 3 (treated with 2 mg/kg of colchicine).

In the Arthus reaction, complement attracts PMNs to develop vascular injury (Cochrane & Dixon, 1978). Specific removal of PMNs by nitrogen mustard treatment prevents accumulation of PMNs and development of vessel wall injury (Cochrane, Weigle & Dixon, 1959). However, the present study confirms the finding that suppression of inflammation by colchicine in the Arthus type reaction is attributable to the inhibition of PMN migration to chemoattractant but not to the decreased number of PMNs nor to the prevention of formation of immune complexes around blood vessels.

It has been shown that migration of PMNs towards a chemotactic source is the only PMN activity affected by colchicine in therapeutic doses (Wallace, Omokoku & Ertel, 1970). Such effects of colchicine may in part be due to its ability to bind to microtubules (Malawista, 1968). It seems reasonable to speculate that the interference with microtubular functions of PMN following the treatment with colchicine may result in immobilization of PMN *in vivo*. The decreased infiltration might be responsible for the prevention of vascular injury and the prolonged deposition of complement in the vessel walls, which we speculate to be the mode of action of colchicine in the Arthus reaction.

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Response of alopecia areata to DNCB: influence of auto-antibodies and route of sensitization

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SUMMARY

The effects of treatment with topical 2,4-dinitrochlorobenzene (DNCB) were observed in fifty-one patients with alopecia areata of at least 9 months duration. Patients were sensitized either by the application of 500 μg of DNCB in acetone to the forearm, or by painting affected areas of the left side of the scalp with a 1% solution. A mild to moderate dermatitis was maintained by weekly applications of DNCB.

When the sensitizing dose was applied directly to the scalp, significantly more patients showed poor reactivity as judged by the eczematous response obtained, although the two routes of sensitization had comparable effects upon regrowth of hair.

Hair regrew significantly more frequently in females. The likelihood of regrowth was reduced in patients with hair loss of long duration and in those with immunological disturbances such as auto-antibodies or low T lymphocyte numbers and responses. The relationship of these factors to the disease and to the response to DNCB treatment is discussed.

There are a number of reports that induction of allergic contact dermatitis with dinitrochlorobenzene (DNCB) in areas of scalp affected by alopecia areata may result in regrowth of hair (Happle & Echternacht, 1977; Daman, Rosenberg & Drake, 1978; Breuillard & Szapiro, 1978; Warin, 1979). All workers agree that results of treatment with DNCB are variable both in the number of patients who respond and the extent to which regrowth occurs. Factors which influence the outcome of this treatment have not been defined, although patients with disease of short duration tend to show better responses while those with a personal or family history of atopy or auto-immune endocrine disease do worse (Breuillard & Szapiro, 1978).

Different workers have sensitized patients at different sites and there are conflicting observations concerning the ability of patients with alopecia areata to express sensitivity to DNCB. It has been claimed that the therapeutic effect of DNCB in some way reflects the involvement of the immune system in the pathogenesis of alopecia areata (Happle & Echternacht, 1977). In previous studies, these patients were found to have disturbances in their immune system such as an increased prevalence

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of auto-antibodies and decreased T lymphocyte numbers and responses *in vitro* (Friedmann, 1981a,b).

The present investigation was performed to see whether the ability of DNCB to stimulate regrowth of hair in alopecia areata is related to underlying immunological disturbances or the route of sensitization.

PATIENTS AND METHODS

Fifty-one patients were studied of whom twenty-two females and sixteen males had total loss of hair (Table 1). In all patients, hair loss was present for at least 9 months and none showed signs of regrowth. Auto-antibodies to thyroid antigens, gastric parietal cells, smooth muscle, mitochondria, reticulin, nuclear constituents and rheumatoid factors were looked for in all patients, and T lymphocytes were counted and their responses *in vitro* to standard microbial antigens (Tuberculo-protein PPD, Varidase and *Candida albicans*) were measured in some. T cells were identified by formation of rosettes with sheep erythrocytes and responses to antigens were measured by incorporation of tritiated thymidine after 6 days culture. 'Low responses' were defined as less than 1000 counts per min (c.p.m.) in stimulated cultures compared with mean values for healthy control lymphocytes cultured with PPD, Varidase and *C. albicans* of 7070, 5300 and 7700 c.p.m. respectively, (Friedmann, 1981b).

TABLE 1. Patients with alopecia areata treated with DNCB

	Number	Total alopecia	Age (years ± s.d.)	Disease duration (years ± s.d.)	Treatment duration (months)
Female	31	22	33·9 ± 17 (12–72)*	7·6 ± 7·4 (0·75–25)	6·4 ± 3·5
Male	20	16	32·7 ± 12 (16–54)	6·6 ± 9·1 (0·75–38)	4·9 ± 3

* Range in parentheses.

Induction of contact sensitivity

Sensitivity was produced by either of two routes:

Forearm. DNCB 500 μg (100 μl of a 0·5% solution in acetone) was applied to an area 3 cm in diameter on the volar aspect of the forearm. The area was covered for 48 h. After 2 weeks, patients were challenged with patch tests of 10 μl of 0·125%, 0·0625% and 0·03% DNCB solutions applied to standard, foil-backed felts. The lowest concentration which produced an indurated response was selected for application to the scalp. If no indurated response occurred, the 0·5% solution was applied to the scalp.

Scalp. A 1% solution of DNCB was painted directly onto the bald skin on the left half of the scalp. After 2 weeks, DNCB solution was applied again but the strength was varied according to the reaction produced by the initial application.

Treatment regimen

All patients received treatment to the left half of the scalp only; the right half served as control. A mild to moderate eczematous reaction was maintained by weekly application of DNCB solutions. The

DNCB was applied by the physician and the strength was varied between 0·05 and 0·5% according to the degree of reaction.

Six patients withdrew from the study before they had completed 3 months therapy. Two women found their psoriasis flared up: only on the scalp in one but in many sites in the other. A third woman developed intolerable headaches related to tender lymphadenopathy at the back of the head. Of three men who withdrew, two were miners who could not tolerate an itchy scalp in their conditions of work the third found weekly attendances too troublesome.

Results are presented only for patients who completed at least 3 months of continuous treatment.

Evaluation of responses

Responses were classified as (1) complete regrowth on the treated side, (2) partial regrowth on the treated side—regrowth ranged from a few vellus hairs to good but incomplete growth of terminal hair, (3) no growth on the treated side and (4) equal growth on both sides—a null effect.

RESULTS

Reactivity to DNCB

Of thirty-four patients who received the sensitizing dose of DNCB on the forearm, nine (26%) took more than 4 weeks to show sensitization as manifested by the occurrence of an eczematous reaction on the scalp. Moreover, the eczematous reaction was maintained only by the application of a high concentration (0·5%) of DNCB. By contrast, a significantly higher proportion—eight of seventeen (47%)—of patients sensitized by direct application of a 1% solution of DNCB to the scalp showed this reduced reactivity ($P<0{\cdot}05, \chi^2 = 5{\cdot}01$, 1 d.f.). When the two routes of sensitization were compared, the effects on regrowth of hair were not significantly different and results have therefore been combined.

TABLE 2. Effects of DNCB on alopecia areata

Result	No.	Duration of disease (years ± s.d.)	Auto-antibodies	T cell number	Low T cell responses
Females					
Null*	0	0	0	0	0
No regrowth	8	12·5 ± 8	5 (63%)	0·71 × 10^6/ml n = 4	75% n = 4
Partial regrowth	17†	7·9 ± 6·6	9 (53%)	1·09 × 10^6/ml n = 8	33% n = 10
Full regrowth	3	0·9 ± 2	0 (0%)	ND	ND
Males					
Null*	2	2·5	0	ND	ND
No regrowth	8	9 ± 11	2 (25%)	ND	60% n = 5
Partial regrowth	7‡	6·5 ± 12·8	1 (14%)	ND	0% n = 2
Full regrowth	0	—	—	—	—

* Equal regrowth on both sides.

† In six, hair fell out while receiving treatment. Auto-antibodies were present in all six.

‡ In two, hair fell out while receiving treatment.

ND = Not done.

Auto-antibodies were present in seven of eleven (63%) of the females and two of six (33%) of the males who showed this weak responsiveness to DNCB.

Effects on hair regrowth

Of forty-five patients who were treated for at least 3 months, only three (all female) showed complete regrowth of hair on the treated side of the scalp. They all had partial hair loss only, and full regrowth developed on the untreated side as well after several months of continued therapy. Twenty of twenty-eight females (71%) and seven of seventeen males (41%) showed partial regrowth of hair in response to treatment with DNCB (Table 2). The difference in effect between the sexes was significant ($P<0{\cdot}05$, $\chi_2 = 4{\cdot}04$, 1 d.f.).

In female patients there was a clear trend of association between hair regrowth and duration of disease activity (Table 2). Thus, patients in whom complete regrowth occurred had disease of shortest duration while those in whom no regrowth developed had disease of longest duration. The likelihood of regrowth was less in patients with auto-antibodies, or reduced T cell numbers or responses (Table 2). The three women who developed complete regrowth of hair lacked auto-antibodies, while of eight who showed no regrowth, five (63%) had auto-antibodies. Of seventeen females who developed partial hair regrowth, six relapsed during treatment; auto-antibodies were present in all six.

Male patients showed poor hair regrowth in response to treatment with DNCB. None developed full regrowth on the treated side alone; the two who had complete regrowth did so equally and simultaneously on both sides (Table 2). Those patients who showed some regrowth had disease of shorter duration than those in whom no regrowth occurred. As for females, regrowth occurred less often in those with low T cell responses. Two males relapsed during treatment.

DISCUSSION

These observations confirm that DNCB treatment induces regrowth of hair in some patients with alopecia areata. The extent of regrowth is variable and occurs more often when hair loss is partial and of short duration; that is in the milder forms of the disease in which spontaneous remission might be expected. The present findings also show that hair regrew more often in females and was inversely associated with the presence of auto-antibodies and diminished T lymphocyte responses and numbers. These disturbances in the immune system of patients with alopecia areata have been found to correlate with extent of alopecia, particularly in females (Friedmann, 1981b). Breuillard & Szapiro (1978) also noted a reduced likelihood of hair regrowth in patients with features of auto-immune endocrine disease in their personal or family histories.

Although the test system showed local effects of DNCB, it could not distinguish a systemic effect from spontaneous remission. For example, the women who developed complete regrowth of hair on the treated side later showed full regrowth on the untreated side. In addition, two men showed simultaneous and equal regrowth on treated and untreated sides. There was no inflammation of the untreated areas and it seems improbable that local spread of DNCB had occurred. It may be that DNCB treatment exerted a systemic as well as a local effect but the onset of spontaneous remission cannot be excluded.

It seems unlikely that the action of DNCB is by a simple irritant effect, since Büchner, Echternacht-Happle & Happle (1978) reported that an irritant dermatitis induced with 10% sodium lauryl sulphate failed to induce regrowth of hair in fourteen of fifteen patients. The effects of contact hypersensitivity are not dependent upon a specific pharmacological effect of DNCB since contact dermatitis induced with an unrelated compound, squaric acid dibutyl ester, has been reported to be just as effective

(Happle *et al.*, 1980). It appears to be the immune mechanisms activated by the contact hypersensitivity that play a part.

It has been argued that the allergic reaction attracts stimulated T cells into the treated area of skin, facilitating the clearance of unidentified antigens such as viruses (Daman *et al.*, 1978). It has also been proposed that contact dermatitis augments suppressor cell activity that could non-specifically inhibit immune mechanisms presumed to be responsible for the curtailment of hair growth (Happle & Echternacht, 1977). However, when the functions of T lymphocytes are disturbed as indicated by the presence of auto-antibodies, low T cell numbers and responses, then T cells are also activated less well by DNCB and are thus less effective in either role. This is further suggested by the poor inflammatory response evoked by DNCB in 50% of patients with auto-antibodies.

The difference in allergic response to DNCB when sensitization was induced directly upon diseased areas of scalp rather than the forearm was surprising. It may be due to normal regional variation in susceptibility to sensitization or elicitation, or it may further represent the immune abnormality of the disease.

Finally, although DNCB caused some regrowth of hair, particularly in patients with disease activity of short duration, it does not appear to offer much hope as a therapeutic measure in those with greatest need—patients with long standing, extensive hair loss.

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Steroid sulphatase activity in the skin biopsies of various types of ichthyosis

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SUMMARY

A simple method was developed for the determination of steroid sulphatase activity in a skin biopsy for routine use. In this method, 6 mg of minced tissue is incubated in 1 ml of Krebs-Ringer bicarbonate buffer with radioactive dehydroepiandrosterone sulphate (20,000 c.p.m., S.A. 1.1 Ci/mmol) for 4 h. After the incubation the liberated unconjugated dehydroepiandrosterone and its possible metabolites are separated from the conjugated compound by extraction with ethyl acetate-ethyl ether 10:90 (v/v). The organic phase is counted in a scintillation counter. The results are expressed as c.p.m. values per 6 mg tissue wet weight.

Steroid sulphatase activity was measured in skin biopsies from nine control subjects and from thirteen patients with various types of ichthyosis. In the different groups studied, the ranges of the c.p.m. values were as follows: controls ($n = 9$) 377–1802; X-linked ichthyosis ($n = 5$) 140–214; ichthyosis vulgaris ($n = 5$) 607–1320; ichthyosiform erythroderma ($n = 2$) 2146–2214; lamellar ichthyosis ($n = 1$) 2185. The reagent blank varied from 180 to 259 c.p.m. In X-linked ichthyosis the c.p.m. values were always less than in the corresponding reagent blank, indicating that no enzyme activity was present in the tissue. In other types of ichthyosis, steroid sulphatase activity was normal. The method described is easy to accomplish in any clinical laboratory where scintillation counting is possible.

It has been shown that patients with X-linked ichthyosis have steroid sulphatase deficiency in their cultured skin fibroblasts (Shapiro *et al.*, 1978a). For routine purposes, cell culture systems are relatively laborious and often impossible to develop in small laboratories. In order to produce a simple method to demonstrate steroid sulphatase deficiency, we earlier investigated if the concentrations of different blood steroid sulphates reflect the steroid sulphatase activity in skin fibroblasts (Ruokonen *et al.*, 1980). The steroid sulphate blood concentrations were not statistically different between control subjects and ichthyotic patients, including the X-linked type. Hence, it was evident that the steroid sulphatase deficiency may be demonstrated only in particular cells, like skin fibroblasts. In this study

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we describe a simple method to determine steroid sulphatase activity from a skin biopsy, which may be used for routine purposes. The steroid sulphatase activity was determined from nine normal subjects and from thirteen patients with different types of ichthyosis.

MATERIAL AND METHODS

Patients and control subjects

A total of thirteen patients with various types of ichthyosis were studied. Each patient was examined by a dermatologist and their disease was classified on the basis of clinical appearance, histological criteria and family history. Five patients were diagnosed as having X-linked ichthyosis, five as having ichthyosis vulgaris, two as having ichthyosiform erythroderma and one as having lamellar ichthyosis. Control subjects were healthy men (4) and women (5).

Tissue samples

Punch biopsies of the skin (diameter 4 mm) were taken from the upper extremities under local 1% lidocaine (w/v)-0·001% adrenaline anaesthesia. Samples were usually taken from affected skin areas but also in some cases from healthy skin areas. The depth of the biopsies was to the subcutaneous tissue, which was removed from the samples, and the epidermis and dermis were taken for the determination of steroid sulphatase activity. For histological analysis the samples were routinely stained with haematoxylin and eosin. Skin biopsies (10–20 mg) were stored at minus 20°C until the steroid sulphatase activity was determined within one or two weeks of sampling.

Steroids and chemicals

The [7α-^{3}H]-dehydroepiandrosterone sulphate (S.A. 1.1 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, U.K. Before use, a 5 ml volume of the aqueous solution of the steroid sulphate was extracted twice with 10 ml of ethyl acetate-ethyl ether 10:90 (v/v). Because of the spontaneous hydrolysis of the dehydroepiandrosterone sulphate the extraction procedure was done once per 3–4 months. Fifty microlitres of the aqueous phase (20,000 c.p.m.) was used for the determination of the steroid sulphatase activity. Ethyl acetate and ethyl ether were twice distilled. Other reagents were of analytical grade.

Determination of steroid sulphatase activity in skin biopsies

Skin samples were cut into numerous pieces with a sharp scalpel in order to obtain a homogeneous mince and 6 mg of the sample was incubated in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7·4) containing 20,000 c.p.m. dehydroepiandrosterone sulphate. Reagent blank tubes, including the substrate, were incubated alongside the reaction tubes. Incubations were performed in a water bath at 37°C for 4 h using air as the gas phase. After incubation the samples and blanks were extracted three times with 0·5 ml of ethyl acetate-ethyl ether 10:90 (v/v). The organic phases containing the liberated dehydroepiandrosterone and its possible metabolites were combined, dried and counted in a liquid scintillation counter (LKB-Wallac 81000).

RESULTS

In Table 1, steroid sulphatase activity in minced skin biopsies is expressed as c.p.m. values of the reaction products, unconjugated dehydroepiandrosterone and its possible metabolites, per 6 mg tissue wet weight after 4 h incubation in Krebs-Ringer bicarbonate buffer. In control subjects and in patients with different types of ichthyosis the ranges of the c.p.m. values were as follows: controls (five women,

TABLE I. The c.p.m. values of unconjugated steroid metabolities after 4 h incubation in Krebs-Ringer bicarbonate buffer with 6 mg minced skin biopsies and 20,000 c.p.m. dehydroepiandrosterone sulphate. The corresponding reagent blank values are also given

Controls and patients	Sex	Skin	Unconjugated steroids (c.p.m.)	Reagent blank (c.p.m.)
C_1	F	Healthy	792	207
C_2	F	Healthy	1802	180
C_3	F	Healthy	400	207
C_4	F	Healthy	886	223
C_5	F	Healthy	553	223
C_6	M	Healthy	1156	248
C_7	M	Healthy	641	215
C_8	M	Healthy	377	215
C_9	M	Healthy	702	215
X_1	M	Affected	200	223
X_2	M	Affected	200	223
X_3	M	Affected	214	215
X_4	M	Affected	178	207
X_4		Non-affected	182	207
X_5	M	Affected	140	180
V_1	F	Affected	910	223
V_2	F	Affected	843	223
V_3	F	Affected	1320	215
V_4	F	Affected	607	207
V_5	M	Affected	656	223
E_1	M	Affected	2214	259
E_1		Non-affected	779	259
E_2	F	Affected	2146	259
L_1	F	Affected	2185	215

C = Control subjects, X = X-linked ichthyosis, V = ichthyosis vulgaris, E = ichthyosiform erythroderma, L = lamellar ichthyosis, M = male, F = female.

four men) 377–1802; X-linked ichthyosis (n = 5) 140–214; ichthyosis vulgaris (n = 5) 607–1320; ichthyosiform erythroderma (n = 2) 2146–2214; lamellar ichthyosis (n = 1) 2185. The corresponding reagent blank values have not been subtracted. In the different types of ichthyosis, steroid sulphatase activity was normal except in X-linked ichthyosis where the c.p.m. values were always less than in the corresponding reagent blank, indicating that no enzyme activity was present in the biopsies. Usually the samples were taken only from affected skin areas. In one patient with X-linked ichthyosis and one with ichthyosiform erythroderma, samples were also taken from non-affected areas. As expected, no enzyme activity was noticed in X-linked ichthyosis, but in ichthyosiform erythroderma repeated determinations always showed clearly higher enzyme activities in affected skin compared with non-affected skin. No difference was seen in steroid sulphatase activity between women and men.

DISCUSSION

In certain cells, steroid sulphatase is located in the microsomes (Sandberg & Jenkins, 1969) where it can hydrolyse inactive steroid sulphates to biologically active unconjugated hormones. However, the physiological importance of this enzyme in different cell types is still obscure. Because steroid sulphatase is bound to cell particles, it cannot be determined in body fluids such as plasma. Our earlier attempt to demonstrate steroid sulphatase deficiency by determining blood steroid sulphates was unsuccessful (Ruokonen *et al.*, 1980). Blood steroid sulphate concentrations were not significantly different in control subjects and in patients with X-linked ichthyosis. The possible enzyme deficiency might, on the other hand, be shown in certain tissues or cells, like fibroblasts. Shapiro *et al.* (1978a) demonstrated steroid sulphatase deficiency in cultured skin fibroblasts from patients with X-linked ichthyosis. Later, Shapiro *et al.* (1978b) showed that patients with X-linked ichthyosis from four countries all had steroid sulphatase deficiency in their fibroblasts. Koppe *et al.* (1978) described deficiency of arylsulphatase C in three infants who later developed ichthyosis of the X-linked type. It is not yet known if steroid sulphatase deficiency is only an associated phenomenon in sex-linked ichthyosis or if it also has connections with the pathogenesis of the disease. The analysis of steroid sulphatase activity from cultured cells is relatively complicated, which limits the application of the method in routine work. The method described in this paper is simple and applicable to all laboratories where scintillation counting is possible.

Dehydroepiandrosterone sulphate, used as substrate in this work, carries its sulphate group at position 3 of the steroid nucleus. The steroid sulphatase is not specific for this steroid sulphate alone but also for other steroids like pregnenolone sulphate which have high affinity for the enzyme and which could be used as substrates in the enzyme assay (Payne, Mason & Jaffe, 1969). Because of spontaneous hydrolysis of steroid sulphates a reagent blank must be included in the incubations. In X-linked ichthyosis the c.p.m. values were always less than the corresponding reagent blank. We suggest that this is because of the action of inhibitors of the spontaneous hydrolysis of steroid sulphates, like phosphate and sulphate ions (Pasqualini, 1967), liberated from minced biopsies. The inhibitors are also present in skin samples other than in those from patients with X-linked ichthyosis but their influence is not demonstrable due to steroid sulphatase activity in these tissues. These results confirm earlier observations (Shapiro *et al.*, 1978a,b; Koppe *et al.*, 1978) where steroid sulphatase deficiency was demonstrated in X-linked ichthyosis. In the other types of ichthyosis studied, the enzyme activity was clearly demonstrable and interpreted as being normal. No difference was observed in steroid sulphatase activity between women and men. In one case of ichthyosiform erythroderma, repeated determinations constantly showed higher enzyme activities in affected than in non-affected skin areas. It remains to be elucidated whether or not this observation is true in all cases of this disease. In conclusion, steroid sulphatase activity can be simply assayed from skin biopsies to differentiate sex-linked ichthyosis from other types of the disease, especially when clinical criteria are unreliable.

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Aquagenic urticaria: evidence of cholinergic and histaminergic basis

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SUMMARY

Two patients with urticaria evoked at the site of contact of skin with water have been studied. Protection of the skin from contact with water by prior application of petrolatum ointment prevented wealing, but removal of the stratum corneum enhanced wealing.

Organic solvents did not themselves evoke wealing, but they enhanced the reaction to subsequent challenge by water.

That the release of acetylcholine is an essential step in the pharmacogenesis of wealing in aquagenic urticaria is indicated by the suppressive effect of locally-applied scopolamine on water-evoked wealing.

Aquagenic urticaria is also associated with elevated blood histamine levels and degranulation of mast cells in the water-challenged skin.

The relationship of acetylcholine and histamine to each other and to contact of water with the skin remains uncertain.

Aquagenic urticaria is a rare form of physical urticaria. The individual lesions are clinically indistinguishable from cholinergic urticaria, although they are not provoked by exercise or by intradermal injection of cholinergic drugs such as methacholine (Mecholyl). They are evoked at the site of contact with water. Pressure, local heating or cooling, or exposure to visible or ultraviolet light does not produce urticaria in patients with aquagenic urticaria.

In this report of two patients with aquagenic urticaria, we provide evidence that local release of acetylcholine is an essential step in the pharmacogenesis of the weal and erythema of this disorder. We have demonstrated the association of histamine release with development of lesions in aquagenic urticaria, though the relationship of histamine to the pathogenesis of the weals is uncertain.

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PATIENTS

Patient no. 1, a 30-year-old female, had suffered from aquagenic urticaria for 1 year. The lesions developed within 10 min of immersion in swimming pool, bath or shower. They faded within 30 min. Cyproheptadine improved the pruritus, but did not stop wealing, and was too sedating. Patient no. 2 was a 14-year-old female who, 18 months previously, developed aquagenic urticaria mainly on the back whilst swimming or bathing, and lasting 15–30 min.

Treatment schedules of weekly doses of antihistamines and a placebo were tried as follows:

(a) phenergan 50 mg at night;
(b) chlorpheniramine 4 mg four times daily;
(c) ascorbic acid 25 mg three times daily;
(d) hydroxyzine 10 mg four times daily;
(e) cyproheptadine 4 mg four times daily.

The number of weals after bathing and the severity of the pruritus were scored daily. All the antihistamines were superior to ascorbic acid, with cyproheptadine and phenergan being slightly more effective than chloropheniramine and hydroxyzine. All the antihistamines were too sedating for routine use. Neither patient was an atopic subject. Both cold and hot water evoked wealing, and the diagnosis was confirmed in each subject by applying a compress soaked in water at 36°C. This was followed by the development of several itchy, small irregular urticarial papules surrounded by an irregular bright red halo at the site of contact with water. Laboratory investigations including haemoglobin, white cell and differential count, serum electrolytes and urea, liver function tests, serum proteins and electrophoresis, the serum complement components of C3 and C4 and serum C1 esterase inhibitor were normal.

METHODS AND RESULTS

Effect of organic solvents

In order to determine whether pure organic solvents would mimic the reaction to water, the response to compresses soaked in absolute ethanol and acetone was studied. Pure acetone and absolute ethanol were applied in symmetrical test sites of area 10 × 10 cm on the back. After 5 min all sites were challenged with water (20 min, patient 1; 30 min, patient 2). The weals in the test areas were counted. The results are summarized in Table 1.

TABLE 1. Effect of organic solvents

Solvent applied	Number of weals Patient 1	Patient 2
Ethanol for 5 min	—	0
Ethanol for 5 min followed by water for 20–30 min	—	2
Acetone for 5 min	0	0
Acetone for 5 min followed by water for 20–30 mins	16	3
Equal mixture of ethanol and acetone for 5 min	—	0
Equal mixture of ethanol and acetone for 5 min followed by water for 20–30 min	—	11
Water for 20–30 min	5	—

Neither acetone nor ethanol caused urticaria when applied locally for 5 min. However, the results with acetone in patient 1, and ethanol + acetone in patient 2, suggested that pre-treatment of the skin with organic solvents enhances the subsequent urticarial response to challenge by water.

Scopolamine

A solution of scopolamine 9% in distilled water was applied for 5 min to a 10 × 10 cm area of the back. Adjacent areas of similar dimensions were treated with 9% scopolamine in acetone. In addition, patient 2 received 9% scopolamine in ethanol on an identical area. Inhibition of thermally-stimulated sweating was confirmed in scopolamine-treated areas by the use of starch-iodine paper. After 5 min all sites were challenged with water (20 min, patient 1; 30 min, patient 2). Weal counts were then carried out in all test areas. The results are summarized in Table 2.

TABLE 2. Effect of scopolamine

Pre-treatment	Number of weals Patient 1	 Patient 2
Scopolamine in water	1	0
Water	5	3
Scopolamine in acetone	3	0
Acetone	16	3
Scopolamine in ethanol	—	4
Ethanol	—	2

In four of the five test areas scopolamine suppressed water-provoked wealing. The fifth test areas showed wealing in both scopolamine-treated and control areas. Taken as a whole, the results indicate that scopolamine pre-treatment suppresses the subsequent wealing response to challenge by water in aquagenic urticaria.

Pre-treatment with petrolatum ointment

The ability of water to provoke wealing in aquagenic urticaria could be prevented by pre-treatment of the skin with petrolatum ointment. In patient 1, four areas of 10 × 10 cm each were selected on the back. Petrolatum was applied thinly but evenly to two of these. One of the petrolatum-treated areas then received ethanol. The second served as a petrolatum control. The third received ethanol alone (no petrolatum) and the fourth was left entirely untreated. After 5 min all areas were challenged by a water compress applied for 20 min. No urticaria developed in the two areas which had received petrolatum. In contrast, the ethanol-treated area (no petrolatum) and the area which had not been pre-treated developed four and two weals respectively.

Effect of removal of the stratum corneum

It seemed possible that an intact stratum corneum is a prerequisite for development of urticarial weals following challenge with water in aquagenic urticaria. Accordingly, the effect of removal of the stratum corneum on the response of skin to water was investigated. Twenty Sellotape strippings were carried out on an area of the back measuring 10 × 10 cm in patient 2. That the stratum corneum had been effectively removed was suggested by the readily visible dermal papillary blood vessels which glistened through the stripped epidermis. Compresses soaked in double-distilled water were applied for 20 min to both this and two otherwise identical areas in which the stratum corneum remained intact. It was found that removal of the stratum corneum did not impair the ability of the skin to respond to challenge with water; on the contrary a greater response was observed in the stripped area (seven weals), compared with the two unstripped areas (two weals each). Thus an intact stratum corneum is not essential for development of wealing in aquagenic urticaria, and may even behave as a barrier to production of wealing following water challenge.

Effect of tap water compared with double-distilled water

In patient 2, challenge with tap water for 20 min in a 10 × 10 cm site produced three weals, compared to two weals after a similar challenge with double-distilled water. Thus tap and double-distilled water appear to be equally effective in evoking wealing.

Evidence for histamine release from involved skin following challenge

In order to detect elevated venous blood histamine levels following challenge with water, the following procedure was undertaken. A cannula was inserted into the right antecubital vein. The back of patient 1 was painted with acetone and the patient was then immersed in warm water. Sequential histamine samples were withdrawn from the antecubital vein for 30 min. Histamine activity in these samples was determined by cascade superfusion assay as described by Bentley-Phillips, Kobza-Black & Greaves (1976). Before bathing, the baseline venous blood histamine concentration was less than 1 ng/ml. After challenge it rose to a maximum of 6·5 ng/ml. That this activity was attributable to histamine was established because the response of the smooth muscle preparation to the venous blood samples was blocked by the specific histamine antagonist mepyramine. The increased histamine activity was not associated with systemic symptoms, but the back and upper arms developed marked erythema and pruritus.

On another occasion, the antecubital vein of patient 1 was cannulated as before, 20 min after the patient had washed her face with water, producing facial pruritus and erythema. The venous blood histamine then rose to 10·5 ng/ml. The arm was then painted in acetone and immersed in water at 36°C for 20 min. A rise of histamine levels in the venous blood to 17·0 ng/ml was noted after 5 min of immersion and 20 min later the histamine concentration was still 15 ng/ml.

A similar study was carried out in patient 2. Despite painting the arm in absolute ethanol followed by immersion of the forearm in water for 15 min, no raised histamine activity was detected in the venous blood (prechallenge venous histamine concentration = less than 1 ng/ml). The challenged forearm developed clinical evidence of aquagenic urticaria, however.

The reproducible development of elevated histamine levels in the venous blood in patient 1 following challenge by water is consistent with the view that histamine release, presumably from skin mast cells, plays a part in the pathogenesis of aquagenic urticaria, in some patients.

Microscopical studies

In both cases punch biopsies were taken from skin in the infrascapular region after infiltration with 1% lignocaine as a local anaesthetic. In case 1, the skin was biopsied soon after immersion in water for 5 min, and in case 2, 10 min after a 30 min application of a gauze pad soaked in distilled water.

The biopsy specimens were immediately cut into smaller fragments and immersed in fixative for subsequent microscopic studies. In case 1, half-strength Karnovsky solution (Karnovsky, 1965) was used; in case 2, the method of Forssmann *et al.*, (1977) was employed, in a slightly modified form. Later the specimens were post-fixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon.

Epon embedded 1 μm thick vertical sections were stained with methylene blue and basic fuchsin

(Huber, Parker & Odland, 1968) and examined by light microscopy. Assessment was made of (a) mast cell population density, using the technique of Eady *et al.* (1979), (b) proportion of mast cells undergoing degranulation, and (c) other histological features.

Mast cells were easy to recognise because of their characteristic morphology and staining properties (Eady *et al.*, 1979). With basic fuchsin the granules stain bright red. Normally, the granules are tightly packed within the cytoplasm of the mast cell. A cell was considered to be degranulating if discharged granules could be seen around the cell in the extracellular space.

The mast cell population density and proportion of cells showing degranulation were 62 cells/mm^2 and 44% respectively in case 1, and 43 cells/mm^2 and 9% in case 2. The normal range of forearm skin is 34–66 mast cells/mm^2 (Eady *et al.*, 1979) with 9–20% showing degranulation (Eady *et al.*, in preparation). Other histological features were not striking in either biopsy specimen. Slight dilatation of mid-dermal blood vessels was present. Oedema was seen mainly in the papillary dermis. There was no perivascular infiltrate of neutrophils or eosinophils and fibrin deposition was not found.

DISCUSSION

Our results suggest that the close morphological resemblance of individual lesions in aquagenic urticaria and cholinergic urticaria is reflected by pharmacological similarities. That release of acetylcholine is involved in the pathogenesis of the weals of aquagenic urticaria is demonstrated by the ability of the specific acetylcholine antagonist scopolamine to suppress the wealing response to water application. Anti-cholinergic drugs have long been known to suppress wealing in cholinergic urticaria (Grant, Pearson & Comeau, 1935; Herxheimer, 1956). Whether the inhibitory effect of anticholinergic drugs is direct, or indirect through suppression of sweating or some other product of sweat gland stimulation, is uncertain. In one of the two patients studied, patient 1, elevated venous blood histamine levels were consistently associated with water-induced urticaria. It is of especial interest that this patient, but not patient 2, showed a high proportion of degranulated mast cells in affected skin suggesting that the histamine released into venous blood in aquagenic urticaria is derived from skin mast cells. The mast cell population density was however normal in both patients. We have repeatedly found an elevated blood histamine concentration during attacks of cholinergic urticaria, usually associated with systemic symptoms (Keahey & Greaves, unpublished results). Neither aquagenic nor cholinergic urticaria responds consistently to antihistamine therapy, although one of the two patients described here, patient 2, noted some benefit from systemic antihistamines. Cholinergically mediated histamine release from mast cells, the mode of action of which is not known, has been demonstrated in the gastric mucosa (Cho & Ogle, 1979) and a similar mechanism may operate in cholinergically activated wealing in skin. Light microscope and electron microscope studies of skin mast cells following water-evoked urticaria in the present studies showed no significant change in numbers of mast cells, and only minimal mast cell degranulation. These negative findings do not rule out water-evoked histamine secretion from skin mast cells as a component of aquagenic urticaria, since only minimal mast cell changes are seen in cold-evoked histamine release of cold urticaria (Eady, in preparation), a disease in the symptomatology of which mast-cell derived histamine is generally considered to play an important part.

The events linking challenges by water with local cholinergic activation and histamine release are unknown. Provocation by water cannot be mimicked by organic solvents, although pretreatment by the solvents potentiates the wealing effect of subsequent challenge by water, possibly because the permeability of the stratum corneum is enhanced by application of the solvents. Conversely, if wetting of the stratum corneum is prevented by prior application of petrolatum ointment, water-provoked wealing is greatly diminished. It has been proposed (Shelley & Rawnsley, 1964) that a component in or on the stratum corneum, probably sebum, plays a key role possibly by interaction with water. Our results argue against this possibility since complete removal of the stratum corneum, far from reducing the severity of the urticaria response to water, appeared to enhance the severity of the reaction. Our findings suggest that whatever the nature of the stimulus-response coupling mechanism, it requires a close association between water and components in the epidermis or dermis.

The present results suggest that water-provoked wealing in aquagenic urticaria involves activation of a cholinergic pathway, possibly leading to histamine secretion from mast cells. Although the mode of activation of this pathway by water is speculative, it appears that factors enhancing penetration of water through the stratum corneum amplify the clinical effects of water contact.

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Pili annulati: electron histochemical studies on affected hairs

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SUMMARY

In this study we have shown that in pili annulati all the normal layers within each cuticle cell are present, and show a normal distribution of cystine. The presence of well ordered macrofibrils, composed of microfibrils embedded in a cystine rich matrix, in both regions of the hair shaft indicates that normal keratinization can and does take place. We have demonstrated the presence of electron-opaque, cystine positive material at the intermacrofibrillar spaces and suggest that the presence of the cortical holes is in part due to insufficient cortical material to occupy the space available, and in part due to the dissolution of the intermacrofibrillar material, possibly by water or other chemical agents, diffusing through an apparently more permeable cuticle.

In 1867, Erasmus Wilson FRS presented to the Royal Society of London 'a specimen of human hair of very remarkable appearance'! In conclusion he commented: 'I therefore refrain from speculating upon the cause of alteration of the healthy and morbid structure presented by this case and restrict myself to the narration of the fact'. The hairs were affected by pili annulati.

Over 100 years later little more is known about the nature of this abnormality though a considerable number of microscopic observations have appeared in the literature. Scanning electron microscopic studies (Dawber, 1972) confirmed the presence of large air spaces within the cortex of the abnormal bands of the hair shaft as well as a cuticular abnormality of 'cobble stone' appearance. The nature of the defect remains obscure but transmission electron microscopic observations (Musso, 1970; Price, Thomas & Jones, 1968) have shown similar cortical air spaces, their formation being attributed to a lack of cortical cell proteins to occupy the space available (Price *et al.*, 1978).

To elucidate further the structural defect present in this disease we have carried out cystine specific electron histochemical studies on affected hairs.

METHOD

Affected hairs were embedded in Spurr's low viscosity resin (Spurr, 1969) and transverse sections, 50 nm thick, were cut through both normal and abnormal bands using an LKB mk III Ultratome. Two staining methods were employed:

(1) Uranyl acetate and lead citrate: for general morphology.

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(2) Silver methenamine (Swift, 1968): as a cystine specific stain showing comparative variations of microfibrillar to matrix protein in the cortex and the distribution of cystine in the cuticle.

The sections were viewed on an Elmiskop 101D, transmission electron microscope.

RESULTS

Cuticle

Normal band. Here the cuticle appeared comparatively normal. All the constituent layers of cuticle cells were present. However, the cell membranes showed very slight but distinct undulations (Fig. 1). Membrane bounded vesicles were seen in greater numbers, particularly in those cells adjacent to the cortex (Fig. 1).

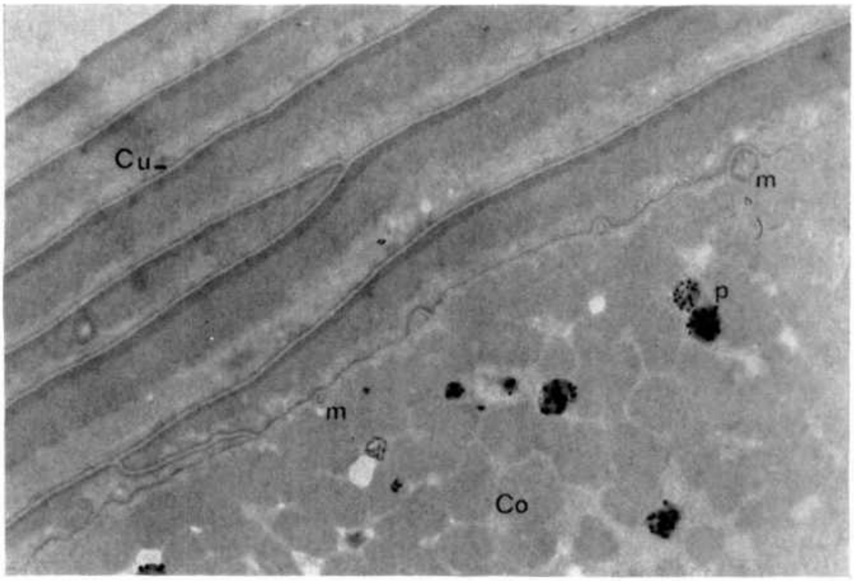

FIGURE 1. Cross section of a normal band. The cuticle membranes (Cu) show slight undulations. Several membrane bounded inclusions (m) can be seen adjacent to the cortex (Co). (p) = Pigment granules. Stain: silver methenamine.

Abnormal band. As in the normal bands the cuticle cells showed a full complement of cell layers. The periphery of the cuticle showed deep indentations (Fig. 2). All the cuticle cells observed showed gross undulations and indentations of the cell membranes, these defects always being reflected in the endo- and exo-cuticles. Numerous vacuoles were present in the endo-cuticle of all the cells though these did not appear to be membrane bounded (Fig. 3). One large inclusion was noted (Fig. 4).

Cortex

In general the cuticular ultrastructure of both the normal and abnormal bands appeared normal. The microfibrillar arrangement showed, in many cases, good hexagonal composition (Fig. 5), with the intermicrofibrillar distance and microfibrillar diameter both normal. The cortical spaces, characteristic of this disease, were only evident in the abnormal band and were seen to occur within cells in the

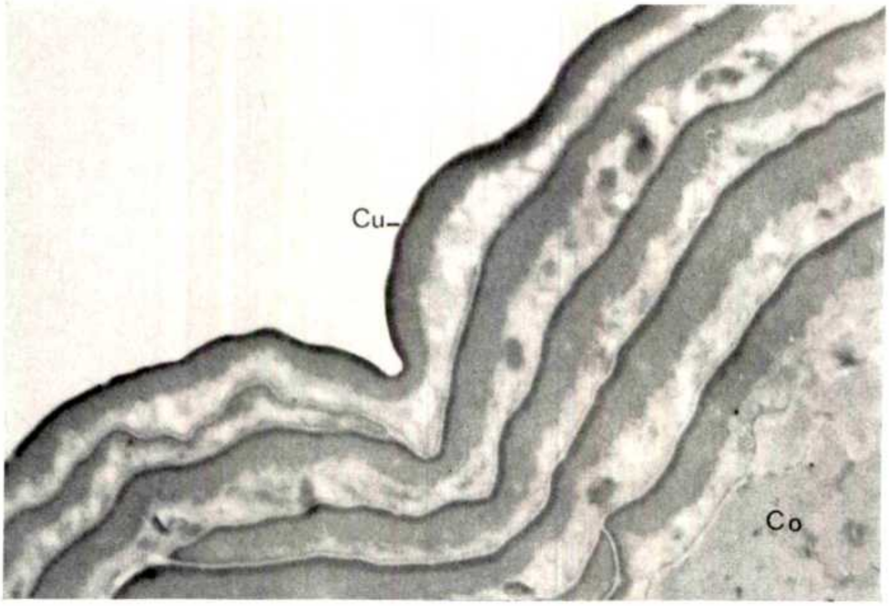

FIGURE 2. Cross section of abnormal band; showing deep indentation of the cuticle periphery (Cu) affecting all the layers of the cuticle through to the cortex (Co). Stain: silver methenamine.

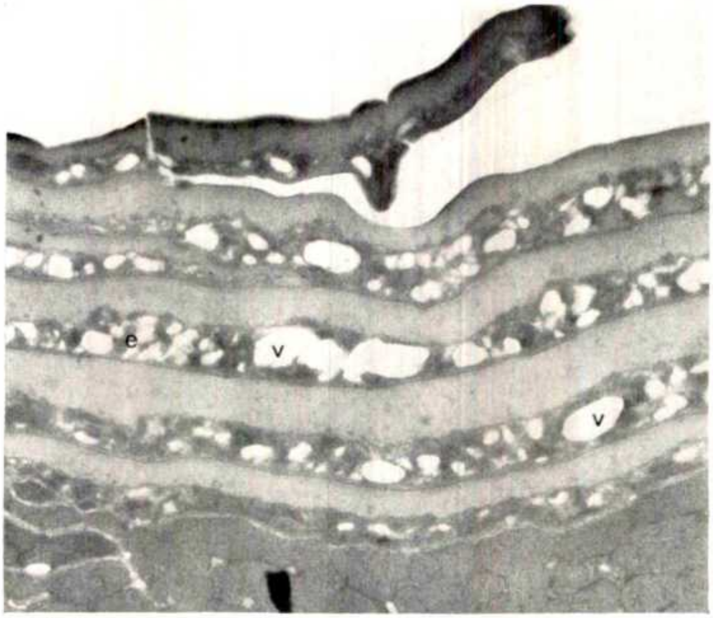

FIGURE 3. Cross section of abnormal band. Numerous vesicles (v) are present in the endocuticle (e) of the cuticle cells. Part of the cuticle can be seen breaking away in the normal process of weathering. Stain: uranyl acetate and lead citrate.

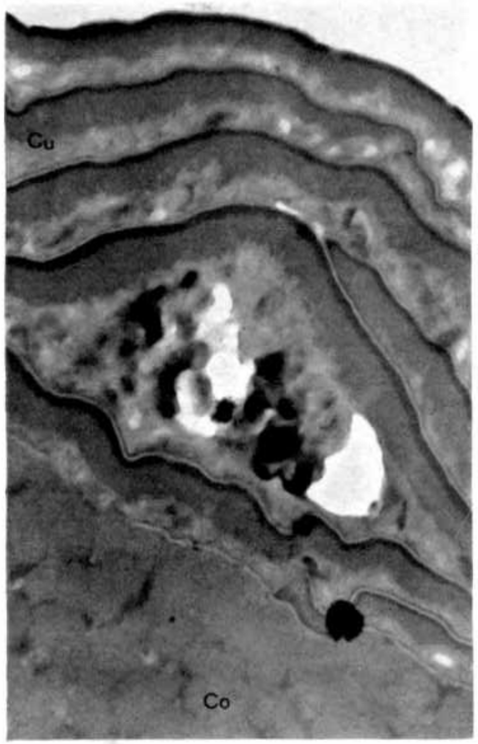

FIGURE 4. Cross section of abnormal band. A large abnormal inclusion is present in the endocuticle of a cuticle cell. Note the undulations in the membranes of the cuticle cells (Cu). Cortex (Co). Stain: silver methenamine.

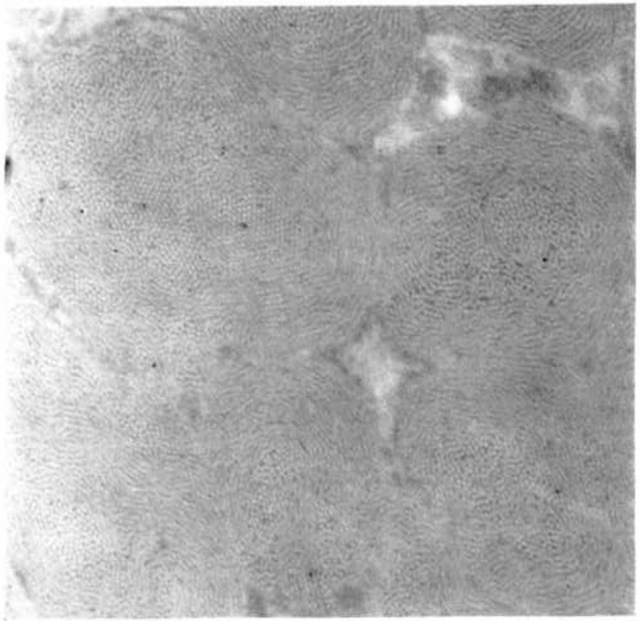

FIGURE 5. Cross section of a region of the cortex showing well ordered microfibrillar patterns of the macrofibrils. Stain: silver methenamine.

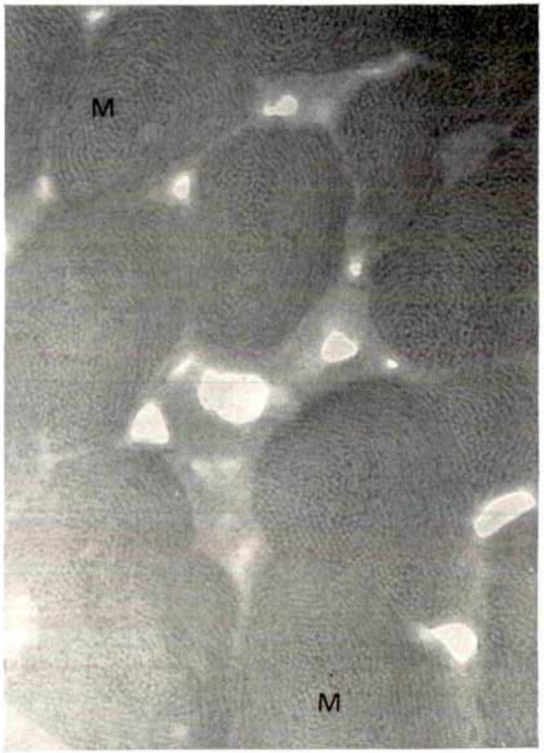

FIGURE 6. Cross section of the cortex showing large spaces between the macrofibrils (M).

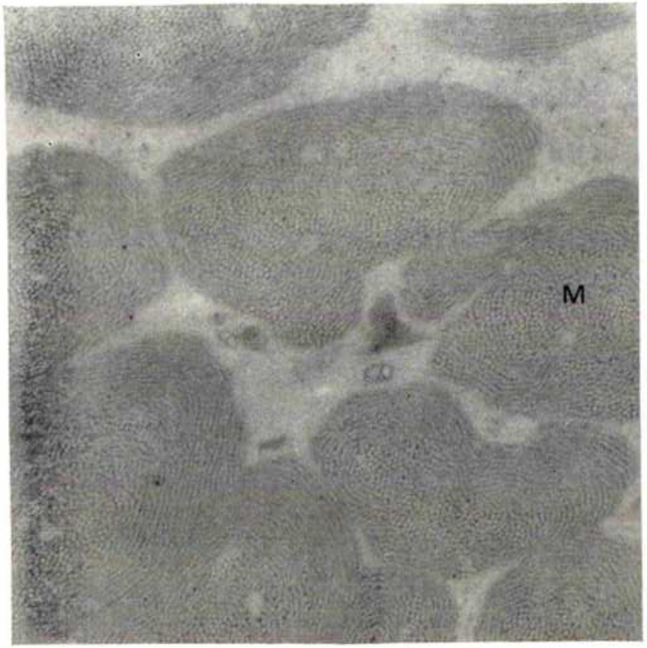

FIGURE 7. Cross section of the cortex showing large areas of electron opaque material between the macrofibrils (M). Stain: silver methenamine.

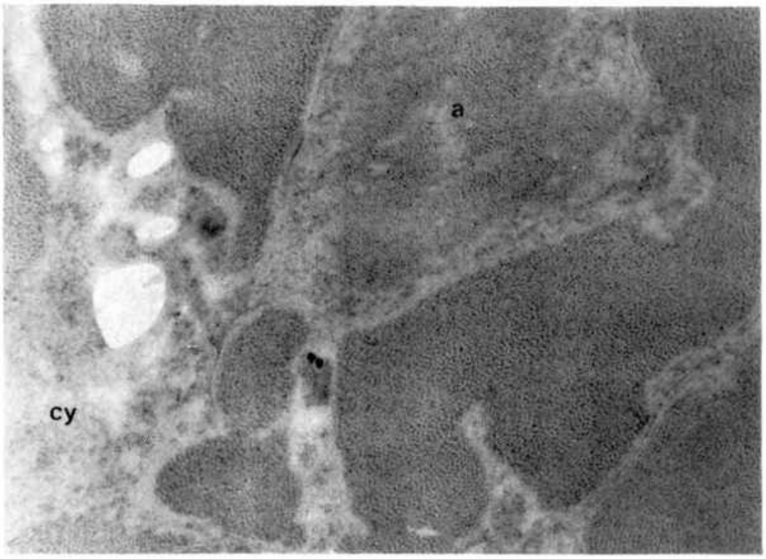

FIGURE 8. Cross section of the cortex showing cystine positive material (cy) in the intermacrofibrillar spaces. The cortical cell (a) appears not to have completely organized into macrofibrillar bundles though many microfibrils are present. The ground matrix in this cell shows postive staining for cystine. Stain: silver methenamine.

spaces between the macrofibrils (Figs 6 and 7). Abnormally large spaces were often seen between the macrofibrils in the form of holes, or sometimes as electron opaque material that stained weakly positive for cystine (Figs 7 and 8). The nuclear remnants of the cortical cells were negative for cystine.

Pigment inclusion at both normal and abnormal bands appeared to be normal.

DISCUSSION

The abnormalities observed in both the cuticle and the cortex were most obvious in the abnormal band regions.

With respect to the cuticle, each cell contained the full complement of cell layers, i.e. a-layer, endo- and exo-cuticle, and all appeared to be completely keratinized. The presence of vesicles within the endocuticle is regarded as abnormal though similar vesicles are sometimes seen in the distal, more weathered ends of normal hairs. The presence of these vesicles throughout the length of the hair shaft may be due to an overall increase in the permeability of the cuticle cells allowing the free passage of a variety of chemical solvents, such as soaps and detergents, to the endocuticle, which is a 'softer' layer possessing few disulphide bonds in comparison with the exocuticle and a-layer.

Indentations and undulations analogous to the surface abnormalities seen under the scanning electron microscope (Dawber & Comaish, 1970) appeared both in the presence and in the absence of vesiculated endocuticular layers indicating that these abnormalities do not occur as a result of expansion or collapse of the vesicular structures. It is most probable that the indentations and undulations occurred prior to the hair becoming fully keratinized since after this stage the hair is subjected to and withstands normal physical trauma (Dawber, 1972). These abnormalities would therefore have to occur between the lower bulb and the zone of keratinization in the follicle and be the result of dynamic forces, possibly induced by differing rates of cell movement between the hair shaft cuticle and the closely opposed cuticle of the inner root sheath.

The holes and spaces observed in cross sections of the cortex were all found to be intracellular, occurring between the macrofibrillar units of each cell. Many of the cells showed an increase in the intermacrofibrillar distance; in the normal hair cortex these spaces are rarely present. Not all the spaces were occupied by holes; many contained cystine positive, electron opaque material. It is possible that these electron opaque areas are the precursors of cortical holes and that the holes develop as a result of material breakdown. This implies that the spaces are not purely the result of insufficient macrofibrillar material being produced to occupy the space available (Price *et al.*, 1968). The presence of cystine-specific silver deposits at the intermicrofibrillar spaces suggests that not all of the available cystine is utilized during keratinization, possibly due to the lack of another component in the process; perhaps the fault in this genetic abnormality is in the supply of keratin building blocks to the follicle.

The fact that the nuclear remnants of the cortical cells remained free of silver deposits, as in normal hairs, indicates the specificity of this technique.

ACKNOWLEDGMENTS

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Patient Management

Dermatological games

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SUMMARY

Doctor/patient relationships may not be optimal in medicine in general and in dermatology in particular. The study of the dermatological consultation has been entirely neglected and game analysis may form a useful basis for further study. It is not possible to know whether better communication with patients leads to better responses to treatment, but there is no doubt that better communication leads to better patient compliance with regard to therapy.

There are many dermatological games. An insight into these games may not only improve doctor/patient communication, but also make the consultation a much more interesting event for the dermatologist. Once the processes involved in the consultation are understood, the way is open for improvements.

The dermatologist should become as interested in the art of practice as he is knowledgeable in the science and clinical features of dermatology.

Most dermatologists spend the majority of their time talking to patients and it is therefore surprising that there are no studies about the mechanics of the dermatological consultation. However, most clinicians would probably agree on the importance of the consultation. Thus, the late Sir James Spence wrote, 'The essential unit of medical practice is the occasion when, in the intimacy of the consulting room, or the sick room, the person who is ill, or believes himself to be ill, seeks the advice of a doctor whom he trusts. This is a consultation and all else in the practice of medicine derives from it' (Spence, 1960).

Recently, there have been several papers suggesting that doctor/patient relationships in medicine are unsatisfactory from the patient's point of view. For instance, a study involving about 100 general practitioners concluded that nearly all doctors had completely failed to adapt their interviewing skills, so that the doctor had become himself a victim of the interviewing style, displaying a remarkable inability to cope with anything but the most mechanical relationship with the patient (Byrne & Long, 1976).

Factors affecting the doctor/patient relationship in dermatology in general, and in psoriasis in particular, were explored by Savin (1970) and the difficulties experienced by the psoriasis sufferer in the community were highlighted by Coles & Ryan (1975). However, a recent survey of psoriatic patients described doctors as 'prescribers' without recognizing or accepting the many other medical, social and psychological problems experienced by their patients (Jobling, 1976). This writer con-

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cluded that changes in doctor/patient dermatological relationships were needed urgently. Other skin diseases have received less attention, but the importance of the doctor/patient relationship during the treatment of acne has been stressed by Reisner (1976). In addition, the formation of self-help societies for eczema and psoriasis may in part be a reaction by sufferers to a non-understanding of the disease by doctors and the problems this creates (Launer, 1976).

Thus, there seems to be some evidence of patient dissatisfaction with the dermatological consultation. An American view of the dermatological encounter was published recently (Waisman, 1979), but methods of looking at dermatological doctor/patient relationships are necessary so that the dermatologist can become as skilled in his relationship with his patients as he is knowledgeable in the science and clinical features of skin disease.

Berne (1964) described game analysis to examine human relationships and this form of analysis lends itself readily to the problem of investigating doctor/patient relationships in dermatology.

According to Berne, human beings ultrastructure their time in three ways, with pastimes, which have no ulterior motive, with 'games', which always have an ulterior motive, and with an ultimate search for intimacy, which is desirable but rarely possible because absolute truth is usually incompatible with the social pressures of living (Berne, 1973). Berne (1973) defined a 'game' thus—'Games are played by people who are afraid of intimacy, either in general or with each other. They are a way of getting close to others and having meaningful transactions without the surrender that intimacy requires'. Thus games can be exploited particularly in doctor/patient relationships and as a way of 'reaching' a patient without becoming emotionally involved.

There are many motives for playing games, but many doctor-orientated games are used to try and comfort the patient. Such games include Reassurance, Prognosis and Denial.

REASSURANCE

Reassurance is a game played by doctors to comfort patients and is one of the commonest consulting room games. However, the medical student is not taught when, why or how to reassure the patient, but learns unconsciously to use this over-prescribed part of the medical armamentarium, often in the face of therapeutic impotence. Although used extensively, and especially by physicians who have been trained only to find and treat organic disease, its effects on patients remain largely unknown and of course it is not possible to subject this form of management to any double-blind clinical trial. Reassurance is usually played by doctors because patients may be ill or feel ill without any demonstrable organic disease and some sort of verbal formula has to be found to save face on all sides. The general practitioner in such instances is usually reassured that the patient's symptoms are functional and the patient is usually merely reassured and often goes away with a strong suspicion that the doctor thinks he is imagining his symptoms.

The value of reassurance must clearly be questioned. There are very few hard facts about reassurance, but in a general practice setting it is known that there is a gap between patients' expectations and what actually happens when the family doctor is consulted (Cartwright, 1967). In this study, advice and reassurance were proffered by doctors much more often than expected by the patients. Reassurance itself was proffered to 41% of the presenting patients, but only 18% were expecting this approach.

Dermatologists are called upon by their patients to reassure them, often about their worries of skin cancer and contagion. We should probably only play Reassurance when our patients want us to do it. The use of this game in other stiuations may represent a retreat from the patient rather than a helpful therapeutic manoeuvre. The therapeutic benefits accruing from reassuring the patient that his particular disease is common, or very rare and that he is unique, have been discussed by Kessel (1979).

TESTS

It is quite common for Reassurance to be supported by a series of tests and this game may be played by dermatologists, patients and by referring family doctors.

The value of diagnostic tests, which usually have no standards fixed for assessment, has been questioned recently (e.g. *Lancet* Editorial, 1979). Moreover, the enormous cost of unnecessary tests has been calculated by Sandler (1979) who found many patients could be managed satisfactorily on the basis of an adequate history and examination. Unfortunately, like Reassurance, Test games can proliferate at an alarming pace. Thus, in the UK between 1967 and 1977, pathological and radiological tests, largely ordered by junior hospital staff, have increased in number by 80% (Abrams, 1979).

One chance in a biochemical profile can lead to a new dimension of diagnostic doubt and thus to a whole series of further investigations, often to the patient's great discomfiture.

One other group of people who are paid to play Tests are research workers and in recent years Papworth (1967) has shown that some of these games may get out of hand and transgress what were thought to be well-recognized ethical boundaries.

Many patients present themselves asking the doctor to play Tests. Patients with urticaria are a common example, often presenting with a conviction that they ought to have skin tests.

Patients are easy to exploit by Test games. Indeed, it has been argued that market demand can be created by the medical profession and industry in the more affluent parts of the world. The response of the general public to computerized axial tomography in the developed world is illuminating in this respect. Many doctors are happy to equate tests with management and the fact that the patient really came initially to be treated can be readily forgotten.

In short, many investigations are performed because of basic clinical insecurity and, ideally, before embarking on investigations there should be a 'pay-off' for the patient in terms of diagnosis, treatment or prognosis.

DIAGNOSIS

Diagnosis is an important game played by both doctors and patients. It is played very energetically in teaching hospitals, so much so that new graduates in medicine may qualify with the belief that diagnosis is the ultimate goal, rather than a starting point in management.

The ability to make a diagnosis depends greatly on the prevailing concept of disease and it must be remembered that concepts of what actually constitutes disease may differ widely (Wulff, 1979). These differences may not only exist between patients and doctors, but have also been demonstrated to some extent between hospital medical staff and general practioners (Campbell, Scadding & Roberts, 1979). Disease has even been regarded as a socio-political concept masquerading as a biomedical one (*B.M.J.* Editorial, 1979).

Conceptual gaps in what constitutes disease may need bridging during the consultation between patient and doctor, and dermatologists must also remember it is possible to be ill without disease and have disease without illness. Is acne a disease, or an invariable accompaniment of adolescence ? Whilst acne may be regarded as a trivial problem by a minority of adolescent sufferers and possibly by unsympathetic medical practitioners, to the majority of those affected it is a very real disease and possibly also a very real illness. Thus, the mature dermatologist must make a complete assessment of his patient's disability related to the social context, rather than just arriving at a mechanistic diagnosis.

PROGNOSIS

It is said that the primary care physician expects three pieces of information in the letter he receives from the hospital specialist namely, the diagnosis, treatment and prognosis. The Prognosis game is intimately related to Diagnosis, Reassurance and Tests. Patients, too, are particularly interested in prognosis and a prognosis game often rightly develops during a consultation with a family with an atopic child, or with a patient with psoriasis.

One way of gaining clinical fame is to arrive at a serious diagnosis with a poor prognosis when the outlook is really good. The patient recovers fully in due course and the client is very grateful.

The most important sub-game in the area of Prognosis is Acceptance. Whilst it is right and proper to investigate patients with disorders such as hirsutes or alopecia, it is unfortunately true that in the majority of patients no treatment will help and the most important facet in the management of these patients is to get the affected individual to come to terms with his or her disease. Incurable and chronic diseases such as psoriasis and eczema may also demand great advocacy in an attempt to get the patient to accept his or her lot. The patient comes to the doctor expecting a cure and is often surprised and dismayed when a cure is not available. In short, the gap between patient expectations and what is possible medically has to be bridged.

TREATMENT GAMES

Placebo game

The concept of a drug as a placebo must never be forgotten. For instance, it is well-known that a significant proportion of acne patients will respond very satisfactorily to the exhibition of a placebo alone (Cunliffe & Cotterill, 1975). This aspect of drug therapy should not be overlooked by the dermatologist. It is important to keep bored patients busy and one method is to ritualize the therapy. It thus becomes possible to justify quite complicated regimens involving two or even three different ointments being used in rotation for strictly prescribed periods of time. Lack of success can again be blamed upon the patient who does not adhere strictly to the ritual that has been laid down. Even changing the colour of the patient's urine can be helpful in the experienced professional neurotic patient.

The Numbers game

The Numbers game consists in telling the patient frankly that perhaps seven out of ten patients improve with treatment X. It follows that the patient may be with the fortunate majority, but there is no guarantee and he may end up in the rather unfortunate minority. This sort of game, like a mutation for a geneticist, always provides a convenient let-out should the doctor's treatment fail.

Allergy

Allergy is a game played popularly by patients, doctors and specialists and also by the media and, in particular, the weekend colour supplements. Most patients want an explanation for their disease and allergy may provide a basis for understanding. The aim of the game is to find the explanation for the patient's symptomatology and may prove to be a very difficult game to handle, particularly if the person playing the game has some medical, nursing or para-medical knowledge. Dentists, community physicians, nurses, laboratory technicians and beauticians can be very difficult to manage when they present playing Allergy. From the dermatologist's point of view, a hard game of Allergy can always by played. All patients are human and a strict adherence to an azo-dye and preservative-free diet, for instance, is a very great rarity. If the patient is not thriving, all the dermatologist may have to do is to get him to admit that he has 'sinned' occasionally.

Torture

Inhuman therapeutics or punitive medicine has been discussed by Dudley-Hart (1978) and it is true that Torture is a game never indulged in consciously now by dermatologists in the U.K. However, the author, in rare moments of objectivity, cannot help but be affected by the screams and squirms of the children he treats with liquid nitrogen. Admitting a patient with mild but troublesome urticaria to a noisy acute medical ward is a more sophisticated form of Torture, and in years gone by it was not unheard of for 'difficult' patients to be treated by intravenous T.A.B.

Bribery and Corruption

The writer was once told by a senior colleague that warts could be 'bought' from children, and the most successful group of children to treat in this way were the children of medical practitioners! Some patients who like to see their dermatologist privately may play a game of Bribery and Corruption. Patients habituated to therapy often bring small bribes (Liddell & Cotterill, 1973).

Folk-Lore and Archaeology

Most people have conscious and subconscious ideas about the skin, its diseases and treatment (Savin, 1970; Burton & Thompson, 1976). For instance, allergy to something, and food in particular, is often linked by patients to urticaria, and so a sufferer from chronic urticaria may readily accept an elimination or an azo-dye and preservative-free diet as part of his treatment. Brittle nails are traditionally associated in the U.K. with calcium lack and a patient with spooned nails may accept easily that he may be short of iron. Thus, the dermatologist may use folk-lore in a positive therapeutic sense.

An important treatment sub-game of Folk-Lore is Archaeology. In this game, patients are told that they are being given a well-tried remedy, and indeed, it may even be a 'triumph' of Victorian polypharmacy. This sort of approach is likely to be more successful in patients with a confidence in old-fashioned remedies rather than new innovations.

New

New is one of the most common therapy games played by doctors and they are often aided and abetted by patients, the press and the pharmaceutical industry. The typical patient is referred by his family doctor to the specialist for something New and very often the patient leaves not disappointed.

Worse Before Better

This game is useful, for instance, in patients with steroid-induced rosacea or perioral dermatitis when local steroids are withdrawn. In this situation the patient may be warned that the skin will get much worse before it gets better. Another variant of this game may be played by the dermatologist when confronted with a patient with very little skin disease; the adolescent with acne, accompanied by a dominant and often anxious mother, comes to mind. This therapeutic situation can be well managed by making the adolescent's acne much worse with an irritant such as retinoic acid; happiness for all parties ensues when this iatrogenic exacerbation is treated with one of the tetracyclines, returning the adolescent to his baseline acne level.

NDCD Syndrome

The Normal Distribution Curve Deficiency Syndrome was described by Shuster (1974), who regarded this disorder as a disabling disease, usually of middle-aged physicians, who produce therapeutic tricks that have worked once. The therapeutic power of the special trick is often reinforced by the fact that it rarely works. This syndrome is pernicious in that it blocks the physician's usual skill in distinguishing chance from cause and effect. This game is played very forcefully by some dermatologists, particularly at regional meetings.

However, the medical anecdote has been defended staunchly (Wooldridge, 1979; Dobson, 1979).

Wooldridge, writing that a properly documented anecdotal account may be neither better nor worse than any other, wrote:

'Dear friends, I plead that you may vote
In favour of the anecdote.
For life itself, in part or total,
Is little more than anecdotal.'

DENIAL

The game of not telling the patient the truth is widely played by doctors, usually for very laudable reasons. Several arguments are normally advanced to support a lie to a patient. Thus, the patient may not understand and may not want to understand or know about his disease, and knowledge of the disease may harm the patient and interfere with treatment (Bok, 1978).

One of the commonest examples of denial in dermatology arises when the patient has skin cancer and the fact that a rodent ulcer obligingly fails to metastasise in the vast majority of patients colludes with the denial-playing dermatologist. However, lying by omission rather than by commission is much more common in the busy consulting room.

It must also be remembered that some patients and doctors, and nurses in particular, when patients, may practise Self-Denial and present late with advanced neoplasms such as malignant melanoma and to other colleagues with other advanced pathologies. This game of self-denial is also played by the frightened, lonely patient with a rodent ulcer who presents very late with an enormous lesion and is usually described by the referring doctor as a 'hermit'.

The patient with dermatitis artefacta presents special problems to the dermatologist. It is thought that confronting the patient with the truth about the pathogenesis of the condition is not helpful (Lyell, 1976). This approach calls for denial in the form of a lie by omission on the part of the attending doctor.

In contrast, most doctors would take a rather dim view of a patient caught lying and adjectives like 'unreliable' are then usually found in the notes. The problem of deception in medical practice has been recently discussed by Naish (1979), but it is interesting that this discussion was centred entirely around patient untruths with no mention of doctor untruths. Patient deception may be important in some clients seeking compensation for their skin disease. A clinical trial involving a comparison of an active drug against a placebo can pose special problems for the physician. How many patients in such trials have been told that they have a 50% chance of receiving a placebo?

Whilst most doctors lie to comfort the patient, they are equally likely to play 'Truth' with an unco-operative patient; for instance, this game is usually played with a patient who may have cancer and refuses any further investigation and the doctor feels justified in putting 'all his cards on the table'. In this type of game the truth is being used to coerce the patient. It is a strange paradox in medicine that truth is often used to threaten and coerce the patient, whilst lies are used for comfort.

FOLLOW-UP GAMES

Follow-up has been the subject of some recent debate (e.g. Loudon, 1976). Whilst there are those who believe that a consultant is there to be consulted and not to collect patients, there are also those who believe in following up their patients, come what may. Such physicians have been labelled 'stamp collectors'. There are many follow-up games that can be played with patients who usually acquiesce readily to this approach.

In a positive therapeutic sense, Gauntlet (cf below) can only be played successfully by Follow-up.

In a negative therapeutic sense, a follow-up appointment may be given to a patient by the doctor when he knows he is to be away.

Dermatologists in training have to gain knowledge of the natural history of the disorders that they are treating and careful follow-up is needed for this. Moreover, many doctors derive at least some job satisfaction from seeing their patients getting better. On the whole, however, the hospital consultant who follows up all his patients tends to be rather despised by his primary care physician colleagues.

Conversely, the patient who fails to keep a follow-up appointment is usually roundly condemned by his medical attendants. It is quite ethical to retreat from one's patients, but it is not ethical for the patient to retreat from the doctor, particularly when the latter has gone out of his way to arrange to see the client for follow-up purposes.

There are very little data about why patients fail to keep follow-up appointments. A recent paediatric study (Cooper & Lynch, 1979) demonstrated that non-attendance at out-patients was likely following an initial referral by casualty without a letter from a family doctor. It was also likely after hospital admission before the first out-patient appointment, and in patients with multiple medical and social problems. The dermatologist should not be angry when his patients fail to return to follow-up, but should wonder why this has happened.

HATE

The dermatologist, like for instance his colleague the rheumatologist, has to deal with large numbers of patients with chronic and often severely debilitating disease. This results in a prolonged and often stressful doctor/patient relationship. In a recent study of a group of patients with systemic lupus erythematosus, Goodwin, Goodwin & Kellner (1979) showed that the patients whom the attending physicians disliked most were either suicidal or had organic brain damage. This group of workers concluded that active dislike on the part of a physician may be a clue to serious psychiatric disturbance in the patient.

ADMISSION

There are several varieties of the Admission game. Patients who are severely incapacitated by any disease, and the skin is no exception to this, may agree readily to come into hospital. However, the doctor may have several other and less laudable ulterior motives in wanting the patient in hospital. Thus, the patient may be an interesting case from which he can gain kudos from colleagues, if properly worked up. The patient may have good physical signs which can be used for teaching the students or for examination purposes. He may be admitted as a guinea-pig to try out a new medication. Both Asher (1947) and Kemp (1964) have stressed that doctors think of healing in terms of the bed. Kemp writes, 'Confronted with real illness our reflex action is to stop the patient working; this gets rid of any ideas he might have had about his personal importance. Then we take away his clothes and put him in a horizontal subservient position; this strips him of his physical dignity, making it clear that he is ill and at our mercy'.

A useful ploy in outpatient practice is to issue the patient with the challenge—'If you are no better in two or three weeks, you will have to come into hospital'. This variety of Gauntlet is usually taken up by the patient, who reappears declaring that he is much better.

Patients who have become institutionalized play admission games as a means of escape from the world. The Munchausen Syndrome may be an example of this. Other variants played by patients include Admission as a means of escape from reality, from prosecution, or even the taxman.

One final advantage of Admission is that the experience may give the patient a better understanding of his condition, when he realizes that there are others that are like him, and some who are worse than he is. Admitting a patient to a noisy, busy general medical ward full of demented geriatric clients is often very helpful as a ploy, particularly in those patients who are weathering the storm, or imagined storm, of their own disorder, rather poorly. This approach, too, may be regarded as a variant of Torture.

DISCHARGE

A sensitive doctor has to play the Discharge game often to ease a patient out of a hospital bed with dignity. Some element of Prognosis is often involved here, in that the doctor has to predict when the patient will be ready for discharge. The patient may also play Prognosis and disagree with this decision, deciding to play Admission instead. In the writer's opinion, the discharge of a patient calls for more care, thought and advocacy than many other of the doctor's tasks. The patient who finds his way back to hospital a few hours after discharge is unlikely to be loved by his medical attendants.

COLLUSION

Collusion is a game played by family doctors, specialists and junior hospital staff. Its aims are laudable in that a second opinion is required to reassure both the patient and the referring doctor. On the other hand, it can be a retreat from clinical challenge and from the patient and it is one way of spreading the clinical load. Collusion is a common game played in teaching hospitals by clever doctors who spend most of their time travelling abroad. Management by committee is never successful and usually adds up to no management at all.

CATHARSIS

Catharsis is a game played by medical practitioners with an interest in the psychosomatic aspects of medicine. It may have positive therapeutic implications and consists of trying to get the patient to express his or her feelings. The feeling of being listened to and understood may be beneficial in itself (Brown, 1976). The positive therapeutic results for the patient may be debatable, but the patient often feels better after the catharsis.

Moreover, Brown & Bettley (1971) showed the beneficial effect of psychiatric treatment in patients with eczema as long as the patient was motivated for this approach.

The importance of insight in prognosis has been stressed (Seville, 1978) and denied (Shuster, 1979). We may summarize that, at worst, listening may help the dermatologist to get to know his patient better.

PEASANT

This game is very prevalent in dermatological circles and it is most difficult to resist playing it. In a classical example, a poor, uneducated, neglected village idiot is brought many miles to see a famous, eminent and versatile clinician. The clinician makes a brilliant diagnosis and advises on management from his ivoried tower, often surrounded by an enormous number of admiring junior staff, medical students, physiotherapists, dietitians and even administrators. The patient goes away confused after the visit with a tube of ointment and a bottle of medicine. Unfortunately, the patient has no idea what to do with the medication, but for the rest of his life owes a burning debt of gratitude to the magnificent doctor for all his time and trouble. In the writer's opinion, a soft game of Peasant is often played whilst managing the skin problems of patients from abroad who speak very little English.

Peasant is often played at dermatological meetings, where patients are presented by one clinician who may ask for advice on the management of his patient. Advice will be forthcoming in great volumes, but it is usually completely out of social context and those proffering it are often doing so more to impress their colleagues than to help the patient.

It is also tempting to play Peasant on one's visits abroad. The courteous host takes his guest on the ward round to see his patients and if one is not very careful, a devastating trail of therapeutic recklessness can be left to commemorate the visit.

The Grand Round and ward rounds rapidly degenerate into Peasant, particularly in large teaching institutions. Steele & Morton (1978) concluded that when a ward round is conducted without regard for the patient's feelings and dignity it can be a potent cause of embarrassment, anxiety and distress and lead to a loss of patient's confidence in the staff and to refusal of treatment or to self-discharge. These workers stressed that to conduct a round when more than two or three people are present, courtesy, experience, appropriate medical knowledge, concentration and a certain amount of stamina are required. The writer is entirely in accord with these views.

YES, BUT

Yes, But is a game fairly commonly played by the bored wives of affluent, busy but tired men. In this game, therapeutic advice is accepted by the patient momentarily and then rejected as being impracticable for one reason or another. The dermatologist will often finish the consultation feeling that his therapeutic avenues have been seriously compromised and that the patient has been manipulating him to her own ends. This game, in the writer's experience, is played much more often in private practice than in NHS practice. A steep bill is the only possible pay-off for the harassed practitioner.

TIME

All doctors have played for time and the natural history of a disease may not always be an enemy.

PRACTICAL APPLICATION OF GAME ANALYSIS TO THE DERMATOLOGICAL CONSULTATION

Let us examine game analysis in the management of one common dermatological disorder, namely chronic urticaria. The patient comes looking for a diagnosis, a cause, treatment and prognosis. Taking the history from a typical patient, worries will be expressed about the possibility of the eruption being an allergy, and a brisk game of Allergy will follow. The patient may come asking for Tests, and this game can be played with gusto. Most patients with chronic urticaria soon express a dislike of tablets and a pleasant game of Folk-Lore, involving dietary control to avoid potential allergens becomes possible. Some patients wonder about their nerves and a game of Collusion with a psychiatrist may present itself. Catharsis is another possible avenue of approach. The patient may be prescribed an antihistamine, which he may not like taking. If this does not work, the Numbers game may be played and the dose doubled or in some other way adjusted. If the patient does not settle with an appropriate elimination diet, a game of Gauntlet may be played and the patient will be told if his skin does not improve, he will have to be admitted to hospital. A hard game of Admission may follow, with Torture in the form of elimination diets, and Provocation Tests. If the patient should be sensitive to azo-dyes or salicylates, the eruption may be made a great deal worse before it improves. A hard game of Discharge can then be played. After this elaborate ritual, the patient is rarely the problem to the doctor that he was before, although the urticaria may still persist and all that remains is to play for time.

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Case Reports

Dyskeratosis congenita. Report of a large kindred

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SUMMARY

This kindred includes six males with dyskeratosis congenita. It is the largest British pedigree so far reported and brings the total number of reported cases to fifty-nine. Our pedigree supports X-linked recessive inheritance and close linkage with the Xg^a locus was excluded. Three previously unreported complications are noted: Hodgkin's disease, adenocarcinoma of the pancreas and deafness. Normal chromosomal stability was found in three patients and immunological studies precluded an early universal defect in cell-mediated immunity.

Dyskeratosis congenita is a rare X-linked recessive condition characterised by skin pigmentation, nail dystrophy, lacrimal duct obstruction and leucoplakia (McKusick, 1978). Its more serious features, however, are bone marrow hypofunction and a high risk of malignant disease. It was first described by Zinsser (1910) and there have to date been fifty-three well documented cases (Gutman *et al.*, 1978). Sirinavin & Trowbridge (1975) have reviewed these cases and noted that onset was usually before puberty with skin or nail changes as the presenting feature.

CASE REPORTS

Case 1 (*III*.24)—*Fig.* 1

The proband is a 25-year-old English male who was well until 10 years of age when he developed skin and nail changes. The skin changes have become more prominent with age. He has had bilateral epiphora and hyperhidrosis of the palms and soles since 18 years of age. Mental and physical development have been normal.

Examination revealed widespread reticulate hyperpigmentation, patchy areas of skin pallor and atrophy, and pigmented striae in the axillary folds and across his back (Fig. 2). The skin pigmentation was most marked on his neck and upper thorax. Nails of all digits were dystrophic but the fingernails were most severely affected (Fig. 3). Conjunctival leucoplakic patches were present but the oral mucous membranes were normal. He did not have palmar hyperkeratosis, splenomegaly or genital atrophy.

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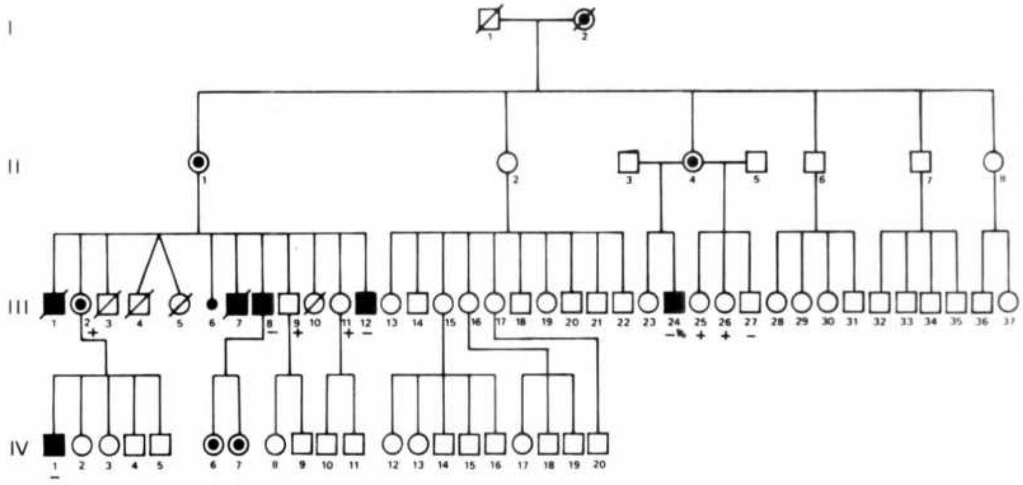

FIGURE 1. Pedigree of kindred. Xg blood group phenotypes indicated + or −.

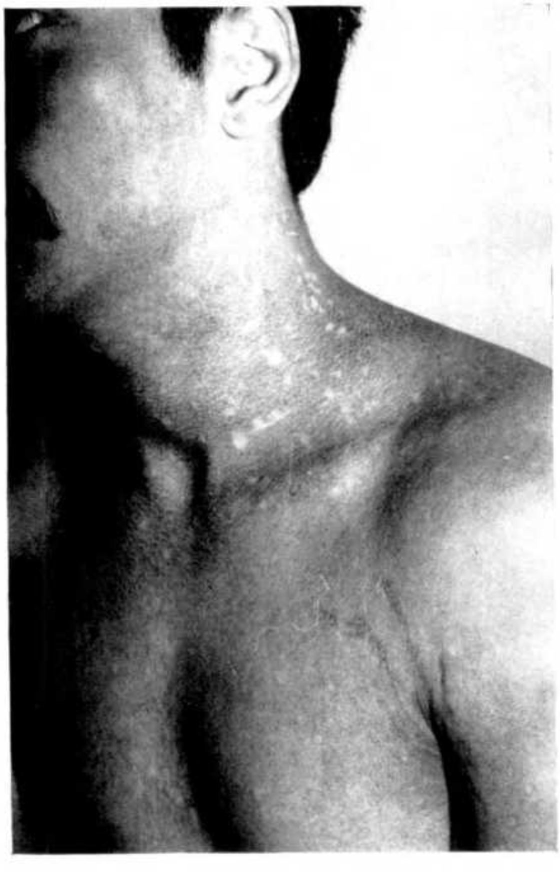

FIGURE 2. Skin changes in the proband at 25 years.

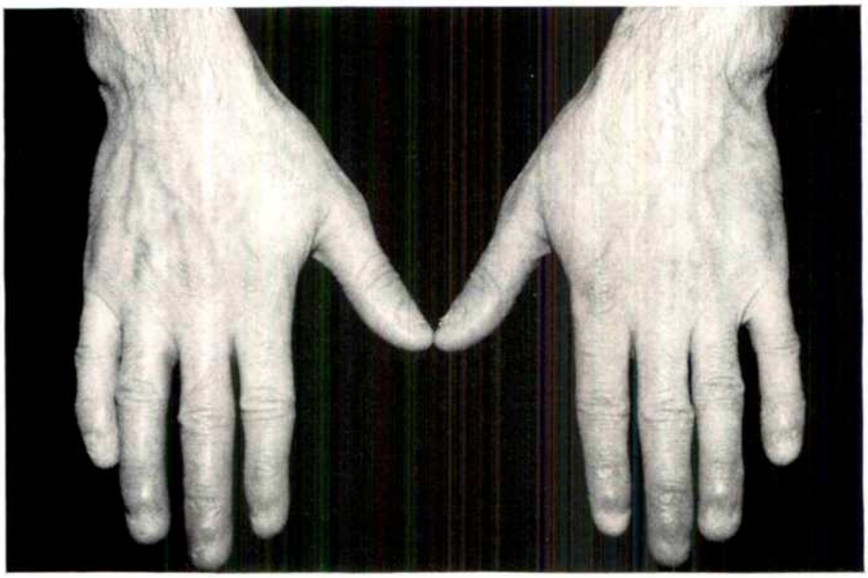

FIGURE 3. Nail dystrophy in the proband.

Haemoglobin was 12 g/dl with haematocrit of 37·1%, mean corpuscular volume 103 fl and a reticulocyte count of 0·7%. Total white cell count was $4{\cdot}9 \times 10^9/1$ with a normal differential. Platelets were reduced at $70 \times 10^9/\text{l}$. Immunoglobulin levels, urinalysis, serum electrolytes, liver function tests, chest X-ray and ECG were within normal limits. Skin biopsy showed an atrophic epidermis with areas of hyperpigmentation in the basal layer. Cultured lymphocytes revealed a normal male karyotype and showed no increase in breakage or structural rearrangements. Mitotic index of his lymphocytes in response to phytohaemagglutinin was normal (5·5%). He was successfully sensitized with dinitrochlorobenzene (Grade 3 response to 0·5% test dose at 8 weeks after initial sensitization).

Case 2 (*III*.1)

This male, the proband's maternal cousin, developed typical skin and nail changes at 10 years of age. He had also epiphora and hyperhidrosis and required multiple blood transfusions for severe pancytopenia. Hodgkin's disease supervened at 23 years and he died when aged 25 years.

Case 3 (*III*.7)

This male had skin and nail changes from 10 years of age but was otherwise well until 29 years when he developed abdominal pain and obstructive jaundice. Laparotomy revealed a widely disseminated mucus-secreting adenocarcinoma of the pancreas and he died shortly afterwards.

Case 4 (*III*.8)

This male is now 29 years old. Skin and nail changes appeared at 9 years and were later followed by epiphora and hyperhidrosis of the palms and soles. Physical and mental development have been normal. He is fertile and has two daughters. Full blood count, immunoglobulin levels, lymphocyte response to phytohaemagglutinin, karyotype and chromosomal stability were normal.

Case 5 (*III*.12)
This male is now 20 years old. Skin and nail changes were again the presenting feature at 9 years of age. He has also epiphora, leucoplakia of the oral mucous membranes, premature greying of the hair and hyperhidrosis of the palms and soles. Physical and mental development have been normal despite bilateral non-progressive sensorineural deafness which has been present since early childhood. Full blood count, immunoglobulin levels, lymphocyte response to phytohaemagglutinin, karyotype and chromosomal stability were normal.

Case 6 (*IV*.1)
This male is now 12 years old. He developed skin and nail changes at 7 years. He has also been troubled with epiphora and recurrent episodes of conjuntivitis. Full blood count was normal.

The maternal grandfather (I.1) had normal skin and nails and died at 70 years of age from a myocardial infarction. The maternal grandmother (I.2) had no stigmata of dyskeratosis congenita and died at 61 years of age of congestive heart failure. They were non-consanguineous and were both born in Liverpool. The carrier females in this kindred were clinically normal and had normal full blood counts.

Colour vision was normal in all living members of this kindred and this X-linked marker was thus not informative. Xg blood groups were determined in the family members where the dyskeratosis congenita gene was segregating. The results are indicated in Fig. 1. Linkage analysis by the Lod score method (Maynard-Smith, Penrose & Smith, 1961) excluded close linkage between the loci for Xg^a and dyskeratosis congenita.

DISCUSSION

The pedigree of our kindred supports X-linked recessive inheritance for dyskeratosis congenita. This seems to be the usual method of inheritance (McKusick, 1978), although one family with autosomal dominant inheritance has been reported (Scoggins *et al.*, 1971). Our study confirms the findings of Gutman *et al.* (1978) that the dyskeratosis congenita and Xg^a blood group loci are widely separated on the X chromosome. Thus linkage is not an aid in genetic counselling in this disorder and this is unfortunate as the carrier females are not at present detectable by other means.

Two of the six affected males in our kindred showed heamatological abnormalities; one with pancytopenia and the other with anaemia and thrombocytopenia. This is in keeping with the findings of Sirinavin & Trowbridge (1975) that haematological abnormalities had been present in approximately half of the reported cases. They noted that pancytopenia was the commonest finding although isolated reduction of only one blood element may occur. The marrow usually becomes progressively hypocellular with fatty and fibrous replacement.

The increased risk of malignant disease in this disorder is supported by the occurrence of fatal neoplasms in two of our six patients before the age of 30 years. Eight of fifty-one (16%) patients in the literature had developed one or more neoplasms by the time they were reported (Sirinavin & Trowbridge, 1975). These neoplasms were all carcinomata and occurred in the skin or mucous membranes. The neoplasms were usually first noted between 20 and 50 years of age. The occurrence of Hodgkin's disease and adenocarcinoma of the pancreas in our patients indicates that this tendency towards malignant transformation affects tissues other than the mucous membranes and skin. The cause of this increased risk of malignant disease is not known but two areas merit further evaluation: chromosomal stability and defective immunity.

Cytogenetic studies have now been performed on twenty-one patients including three in our kindred. Some patients (Scoggins *et al.*, 1971; Morris, 1974), however, have had abnormal chromosomal breakage, but in fourteen a normal karyotype with no evidence of chromosomal instability was found (Ortega *et al.*, 1972; Gutman *et al.*, 1978). Thus the weight of evidence so far is against chromosomal instability as an integral feature of the disease.

A number of immunological abnormalities have been found in patients with dyskeratosis congenita. Scoggins *et al.* (1971) found that three of their four patients failed to show a delayed response to a variety of skin antigens and could not be sensitized by dinitrochlorobenzene. Lymphocytes may also show a defective response to mitogenic stimulation with phytohaemagglutinin (Sirinavin & Trowbridge, 1975). Thymic atrophy may be found at post mortem (Inoue *et al.*, 1973). Gutman *et al.* (1978), however, found normal lymphocyte response to mitogenic stimulation but absence of delayed hypersensitivity to several skin tests. Occasionally some or all of the immunoglobulins may be reduced (Ortega *et al.*, 1972). In all three patients in the present study the immunoglobulin levels were normal and lymphocytes had a normal mitogenic response to phytohaemagglutinin. Further, the proband was successfully sensitized to dinitrochlorobenzene thus indicating intact cell mediated immunity. In this respect dinitochlorobenzene, as it involves known prior exposure, is preferable to skin tests using tuberculin or fungal antigens. These findings indicate that immunological defects are not an early universal feature of dyskeratosis congenita. Faults in cell-mediated immunity may, however, emerge only later in the disease course and then result in the observed increased risk of neoplasia by defective immune surveillance. Serial immunological assessment of these patients should help to clarify this point.

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Perioral pustular eruption caused by *Candida albicans*

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SUMMARY

A 22-year-old female and a 60-year-old male suddenly developed perioral erythematous infiltration with multiple papular and pustular lesions similar to acne conglobata.

Many colonies of *Candida albicans* were cultured from pustules prior to systemic treatment with antibiotics and topical anticandidal treatment.

After healing of the lesions with some scarring, *Candida albicans* could not be cultured.

Severe papulo-pustular 'explosive facial acne' named pyoderma faciale can be seen in young adult women (Plewig & Kligman, 1975), and a variant located on the chin has been described recently (Barker & Gould, 1979).

The present report concerns two healthy patients with a severe perioral eruption, different to common perioral dermatitis and more like pyoderma faciale. In these cases *Candida albicans* was incriminated as an aetiological factor.

CASE I

A 22-year-old healthy English woman was first seen in June 1979 with perioral erythema, papules, pustules and crusts, which had been present for 3 weeks.

The patient had not previously had any skin disease or any other predisposing factors and had not been treated with steroids or antibiotics, either topical or systemic. A punch-biopsy from affected skin showed severe inflammation in the corium with mainly polymorphonuclear leukocytes and some lymphocytes, plasma cells and histiocytes. The inflammation was granulomatous in some areas, but neither giant cells nor necrosis was found.

Selective culture from pustules for mycological examination showed clusters of blastospores and pseudohyphae by direct microscopic examination, and many colonies of *Candida albicans* were cul-

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tured prior to treatment and three times during the initial 4 weeks of treatment. Initially the patient was treated with tetracycline 1 g per day for 2 weeks, during which the lesions progressed with considerable swelling and infiltration and large pustules in the perioral region (Fig. 1). The treatment was changed to erythromycin 250 mg four times per day orally and clotrimazole solution 1% (Canesten) topically, and the skin healed slowly during 6 weeks. With later follow-up visits more discrete papules and pustules in the perioral region were cleared by benzoyl peroxide gel 5% (Benoxid), and only slight scarring in the region was present at the latest follow-up 14 weeks after the initial visit. After the lesions cleared cultures for *Candida* were negative.

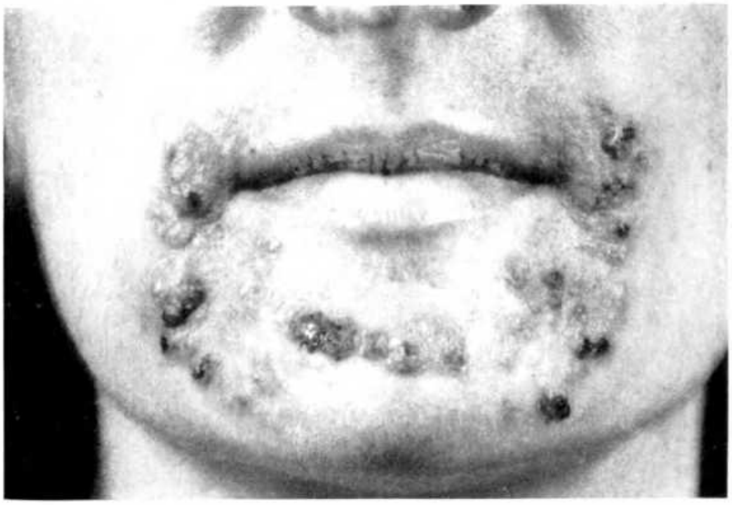

FIGURE 1. Severe inflammation, pustules and nodules after tetracycline-treatment for 2 weeks (case 1).

Bacteriological examinations of material from pustules at the initial visits showed *Staphylococcus albus* or no bacteria on culture.

The patient had no signs of genital or oral candidiasis or other infections. The menstrual periods were regular and no birth control pills or other medicines were being taken. The patient was not exposed to acneigenic agents. Haemoglobin, ESR, leukocyte- and thrombocyte-counts, immunoglobulins, liver function tests, blood sugar, plasma-testosterone, urine 17-ketosteroids and 17-ketogenic steroids were all within normal limits.

Candida skin tests were positive at 48 and 72 h; lymphocyte function tests *in vitro* were normal including normal lymphocyte transformation after stimulation with *Candida albicans* antigen.

CASE 2

A 60-year-old male in good health and without previous skin disease or any other predisposing factors suddenly developed 1 month before examination inflammatory nodules and pustules in the beard area. Sycosis barbae was suggested and the patient was treated with oral penicillin and sodium fusidate (Fucidin) ointment for 3 weeks, but with no effect.

On examination boils and pustules were present in the beard area with purulent secretion and yellow crusts. Lymph nodes were not affected, and there was no fever. Repeated bacteriological examinations were negative, but mycological examination disclosed *Candida albicans* which on culture grew in large colonies.

Treatment with miconazole (Brentan) cream was instituted, and during the following month the skin lesions gradually subsided, leaving small atrophic scars.

ESR, haemoglobin, leukocyte and differential count, serum-creatinine, blood sugar and liver function tests were all within normal limits.

DISCUSSION

Some cases of perioral dermatitis have been ascribed to infection with *Candida* (Bradford & Montes, 1972; Oehlschlaegel *et al.*, 1971). In our cases severe inflammation, pustules and nodules were seen which were different to the tiny papules and pustules seen in perioral dermatitis and more like the facial acne conglobata seen as a sudden eruption in young adult females, named pyoderma faciale (Plewig & Kligman, 1975).

In our two cases the eruption was located strictly to the perioral region and in that respect was similar to the localized pustular eruption on the chin described by Barker & Gould (1979) in females, but in these cases no pathogenic organisms were cultured.

In our cases there was no response to treatment with oral antibiotics, which is in agreement with another pathogenic mechanism than that in perioral dermatitis and acne. The skin changes in our two cases cleared only after topical antimycotics.

It is recommended that bacteriological cultures should be supplemented with mycological examinations in atypical cases of perioral pustular eruptions.

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Dr Else Svejgaard, MD, is kindly thanked for help with candidin skin test and lymphocyte function tests.

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A case of Maffucci's syndrome*

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SUMMARY

A remarkably florid example of a well known, though rare, association of dysplasias is presented in which there is hardly a bone in the skeleton nor a vascular region that has escaped involvement.

Radiographs of the patient are presented and since we shall be commemorating Maffucci's centenary in 1981 it seems appropriate to allow him to 'write' the captions to the illustrations. These captions have been selected from Maffucci's paper, and even though the roentgen-ray was entirely unknown to him at that time his descriptions seem exceptionally appropriate 100 years later

Maffucci (1881) first described the syndrome which afterwards assumed his name as 'Enchondroma with multiple angioma'. His case was that of a married woman aged 40 who had given birth to four healthy sons. She was admitted to hospital because of frequent and severe haemorrhage from an ulcerated tumour on the middle finger of the left hand. In view of the profuse bleeding, and in an endeavour to save her life, her forearm was amputated. All went well for a few days but she subsequently succumbed to pyaemia, and Maffucci thought the infection came from several cases of erysipelas present on the ward at the time.

Maffucci undertook a thorough investigation at autopsy, which he records with such remarkable detail that all his main points have not been improved upon. In order to demonstrate this we have chosen to use, where possible, descriptions taken from Maffucci's own paper as captions for our illustrations.

CASE REPORT

The patient, a male aged 24 years, was first seen in October 1977 for a swollen painful right thigh. There was erythema on the right buttock, in the centre of which could be seen small vesicles thought to be secondary lymphoedema. This picture had been recurring over many months and appeared to derive from an infected ingrowing great toe nail. A swab taken from the toe nail yielded a heavy growth of coagulase positive staphylococcus. All settled on appropriate antibiotics.

The general appearance of the patient is well shown in the illustrations (Figs 1, 2 and 3). He was 122 cm in height and weighed 53·5 kg. He had normal facies, scoliosis on the left side, multiple haemangiomata on the right hand, both legs and feet.

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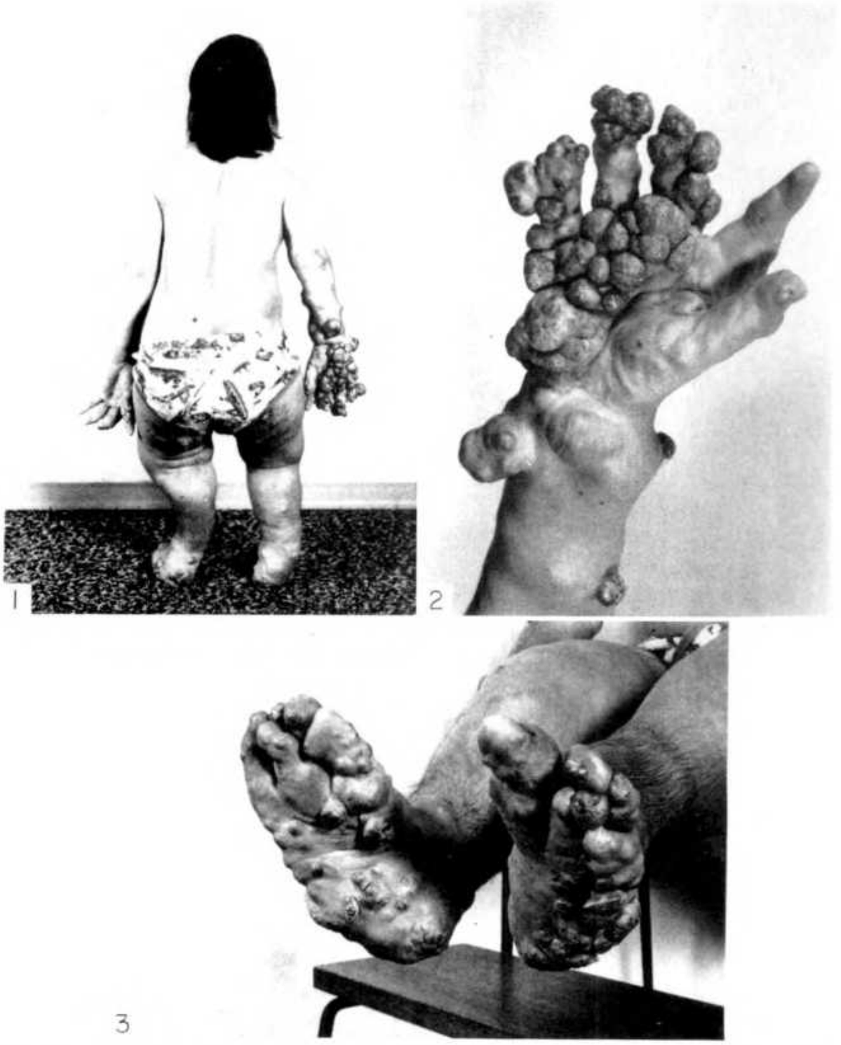

FIGURE'S 1, 2 and 3. The general appearance of the patient with Maffucci's syndrome.

The first tumour had appeared on the middle finger of the right hand at the age of 5 years. He sustained a pathological fracture of the left tibia when he was 12. This had been slow to heal and had resulted in deformity below the left knee.

The patient was well balanced mentally having average intelligence, and despite his disabilities, was of a cheerful disposition with a good sense of humour. He was the youngest of four, his one brother and two sisters being of normal proportions and in good health. His greatest handicap had been due to inadequate footwear. On the whole he had tended to rely on bedroom slippers. These rotted in wet weather, and so he had made several efforts to wear leather boots. This invariably resulted in friction of the toes with skin breakage and infection. He was incapable of using public transport, being unable to climb onto the bottom step of a bus. He overcame this disadvantage by learning to drive a modified car which had adjusted pedals and driving seat. He also learnt to shoot

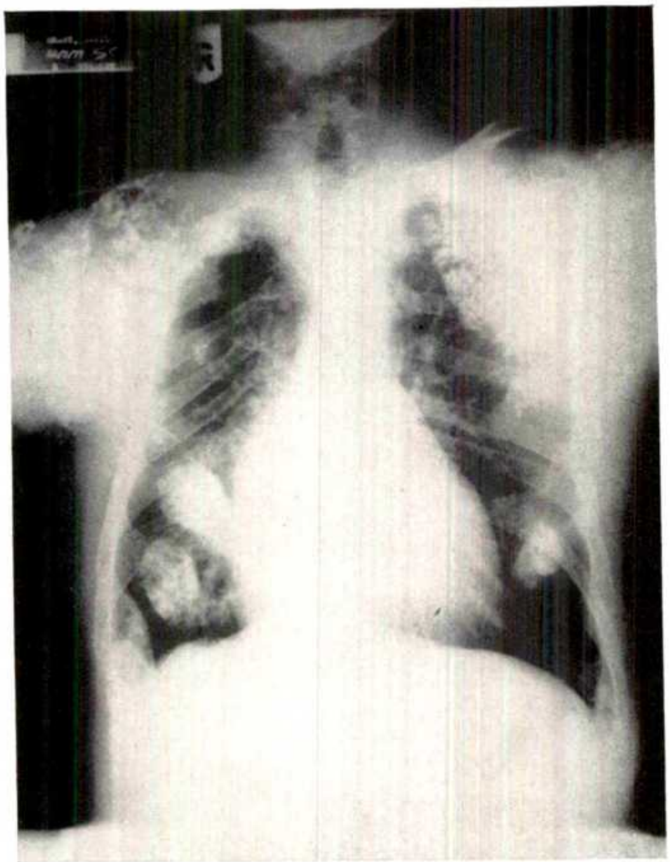

FIGURE 4. Chest. All visualized ribs and shoulder girdle (with sparing of the clavicles) show involvement by enchondromatosis. 'One of the ribs about its maximum curvature presented a tumour the size of a pigeon's egg, completely surrounded by a very thin bony shell and consisting of a very soft cartilage-like substance' (Maffucci, 1881).

with surprising accuracy from his right shoulder, using his right index finger to squeeze the trigger! He walked with a waddle and tired easily.

In view of the repeated episodes of infection it was decided to perform radical excision of both ingrowing big toe nails. At operation the anaesthetist found a haemangioma on the posterior pharyngeal wall above the vocal chords, large enough to prevent intubation, and it proved extremely difficult to maintain an airway.

After the operation the patient made an uneventful recovery. He had boots made and fitted for him at the hospital, and has had no further cellulitis.

In November 1978 he was admitted to hospital for investigation of pain with swelling of the left knee joint, together with haemorrhage from a granuloma over one of the cavernous haemangiomata on the right hand. X-ray of the knee joint showed no evidence of chondrosarcomatous degeneration. The haemorrhage stopped with pressure and the granulamatous lesion healed with the application of 0·5% silver nitrate solution.

From this date on he has been reviewed twice yearly, the last occasion being in September 1980, when his condition did not appear to have altered in any way.

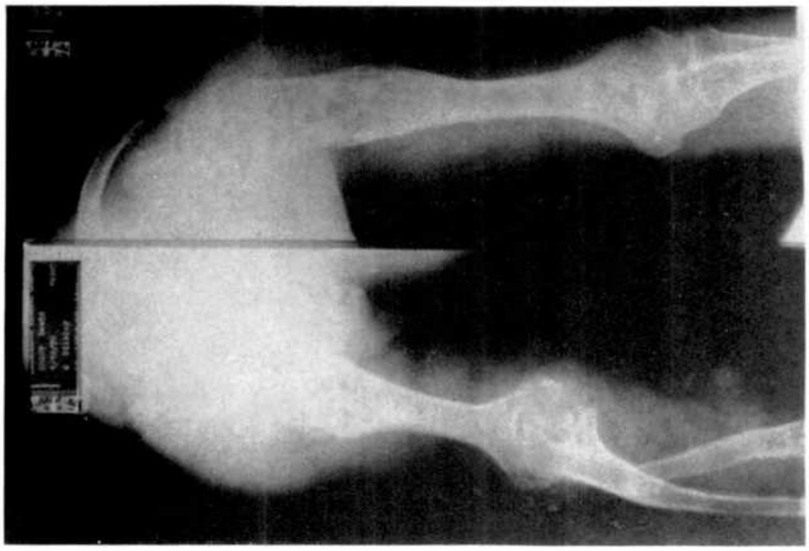

FIGURE 6. Arms. Gross, assymetric shortening of long bones evident, with deformity as a result of growth impairment. 'The head of the humerus showed a normal cartilage cover and down from the interepiphyseal line towards the marrow cavity, the meshes of the spongy bone and the medullary canal contained a hyaline mass, smooth to touch' (Maffucci, 1881).

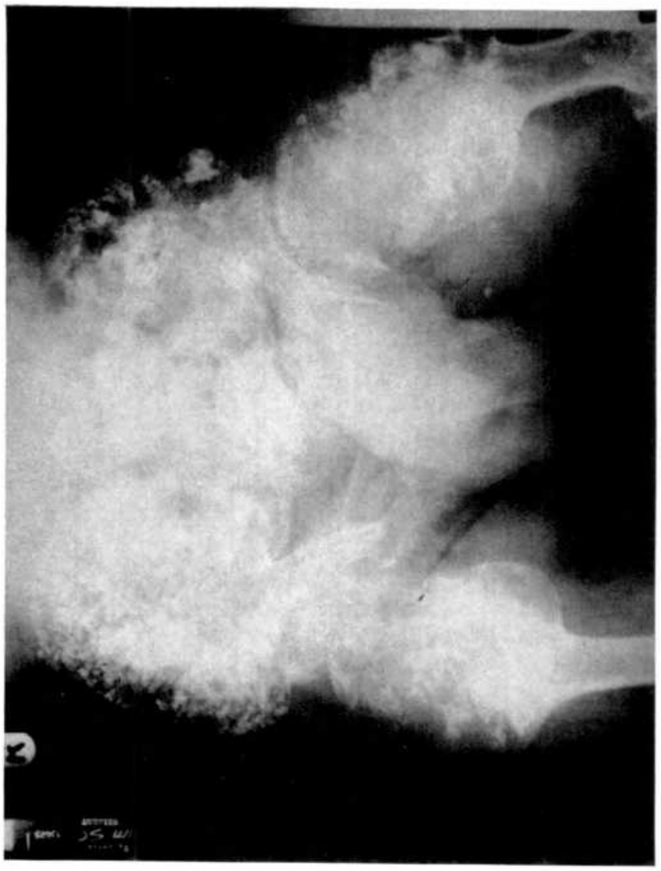

FIGURE 5. Pelvis. Extensive involvement of virtually all visualized bones. Note soft tissue shadows of haemangiomata in the left thigh.

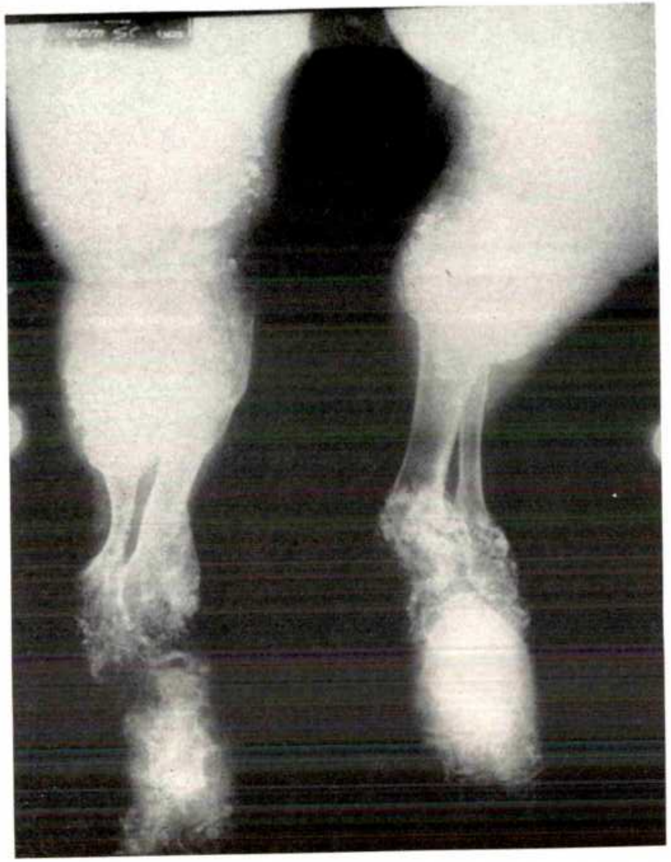

FIGURE 7. Legs. Gross, assymetric shortening of long bones evident, with deformity as a result of growth impairment. 'The lower epiphysis of the left tibia showed large specks of a dark, very soft cartilaginoid material, scattered mainly towards the marrow cavity and towards the interepiphyseal line, where the bone was somewhat enlarged and deformed' (Maffucci, 1881).

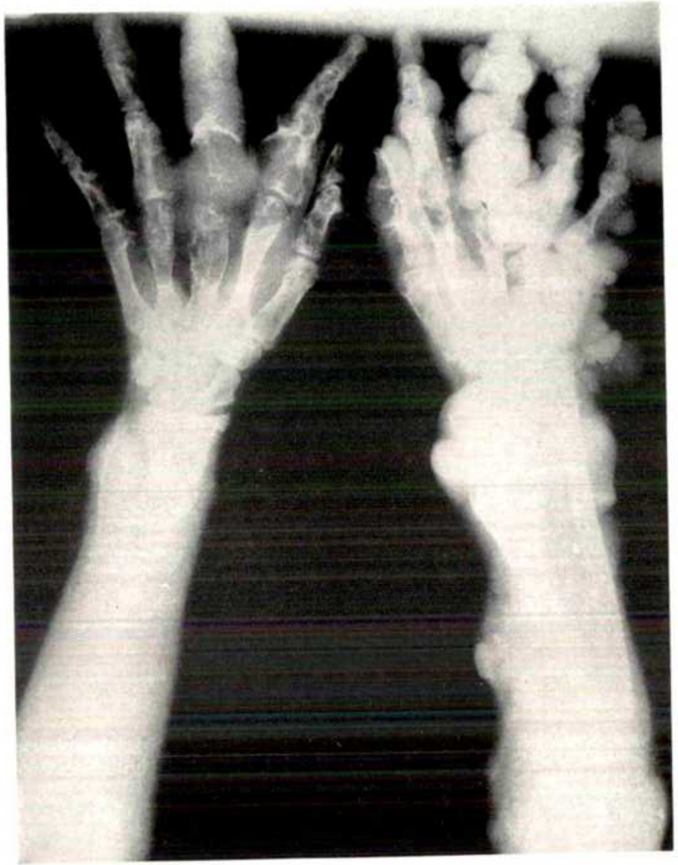

FIGURE 8. Note extensive involvement of all bones by enchrondroma and marked soft tissue masses with calcification. 'The tumour of the hand was covered by skin and subcutaneous tissues and all the metacarpal bones were altered by the neoplasm' (Maffucci, 1881).

DISCUSSION

Maffuci's syndrome is a combination of enchondromatosis and multiple soft tissue haemangiomas that may have phleboliths. It is of interest that one of the dysplasias contributing to the syndrome was first described and named by Ollier in 1900, nearly 20 years after Maffuci's description.

Enchondromatosis or dyschondroplasia results from cartilage failing to undergo the normal process of enchondral bone formation. Rounded masses or columns of uncalcified cartilage are produced within the metaphyses and diaphyses of certain bones, which are invariably shortened. Calcification with healing may occur in adulthood but deformity persists.

Unilateral involvement of long bones, particularly the bones of the hands and feet, is most common but extensive bilateral disease involving flat bones occurs.

The most important complication of this condition is the high frequency of chondrosarcomatous metaplasia with which it is associated (Lewis & Ketcham, 1973).

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British Journal of Dermatology (1981) **105**, 337.

Society Proceedings

BAD Investigative Group Meeting, Cardiff, January 1981

SUMMARIES OF PAPERS

(1) A photographic photometric fluorescent technique used to assess stratum corneum turnover rate and stratum corneum barrier function *in vivo*

A.Y.FINLAY, R.MARSHALL AND R.MARKS *Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff.*

A photographic technique has been developed to measure fluorescence in skin. This has been used to measure stratum corneum (SC) turnover rate and to assess the transfer of topically applied fluorescent agents through the SC *in vivo*. Measurement of fluorescence is made from density measurements of photographic negatives exposed to the fluorescence, which is quantified by reference to a standard fluorescence scale photographed with the site studied.

Dansyl chloride has been used as a fluorescent marker in the assessment of SC turnover time, but subjective assessment of the end point of fluorescence has been difficult. After applying dansyl chloride for 24 h, daily measurements of fluorescence were taken and a precise end point could be calculated.

By applying fluorescent agents topically and measuring the degree of fluorescence at different levels of Sellotape stripping we have been able to measure the movement of topically applied fluorescent agents through the SC *in vivo*. A profile of the diffusion characteristics of dansyl chloride within the SC has been constructed by comparing the degree of fluorescence at strip levels 0, 3, 6, 9 and 12, at times 5, 30, 60, 90 and 120 min.

By applying various topical agents beforehand and repeating this profile we are able to assess changes in barrier function. If DMSO 50% is applied for 20 min before dansyl chloride application, the 60 min profile is profoundly altered, but occlusion of the area beforehand has little effect.

The development of this technique therefore provides a new non-invasive method for assessing SC turnover time at different sites and allows a new method for quantifying changes in barrier function.

(2) Dexamethasone receptors in epidermis

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Although potent topical anti-inflammatory steroids have been widely used in the treatment of many dermatoses during the last 20 years, and although it is generally agreed that the steroid molecule combines with a high affinity cytoplasmic receptor once it has entered the cell, no reports of epidermal steroid receptors have yet appeared.

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Epidermis was removed with a Davies dermatome so that there was minimal contamination with dermis. The tissue was frozen in liquid nitrogen, pulverised in a Braun Mikrodismembrator and then suspended in 0·01 M tris-HCl buffer pH 7·4 containing 10 mM mercaptoethanol.

Portions of the high speed supernate obtained from this suspension were incubated at 4°C with ^{3}H-dexamethasone at a final concentration of 10^{-8}M, and also in the presence of a large excess of competing non-radioactive dexamethasone. The mixture was then treated in one of two ways.

To one portion, charcoal was added to adsorb out unbound dexamethasone. The charcoal was sedimented by centrifugation and the supernatant counted. The presence of non-radioactive dexamethasone significantly reduced the amount of radioactivity remaining in solution, thus indicating the presence of a specific cytoplasmic receptor.

The other portion of the incubation mixture was subjected to electrophoresis in agar, when the unbound steroid migrated to the cathode and the steroid-receptor complex migrated as a single peak to the anode, thus confirming the presence of a specific cytoplasmic receptor for dexamethasone in epidermis.

(3) Investigations into the pathogenesis of monilethrix: a light and electron microscopic study of affected anagen follicles

C.L.GUMMER AND R.P.R.DAWBER, *Department of Dermatology, The Slade Hospital, Oxford.*

Monilethrix hair fractures due to environmental influences frequently before attaining a length no greater than a few millimetres. This, not the beading, gives rise to the established clinical appearance. Work in this department has categorized the intrinsic defect in the surface structure (Dawber, 1977) and the hair cuticle and cortex (Gummer & Dawber, 1980) using scanning electron microscopic and electron histochemical techniques. The specific defects found in these studies are of course history, and to further elucidate the pathogenesis of monilethrix hair breakdown, it is essential to study anagen follicles of affected individuals.

In this study we report light and electron microscopic studies on anagen follicles from four affected individuals.

The abnormalities detected were found specifically in the prekeratinization zone of the follicles. In this region, disorientation of alpha-cortical proteins, invagination of cuticle cells into the cortex, and longitudinal buckling of the cuticle cell membranes of the presumptive hair shaft were all seen. At the sites of these changes, associated abnormalities were present in all three layers of the internal root sheath in varying degree; in particular the Henle layer showed malalignment of individual cells accompanied by increased thickness of the external root sheath.

From our observations we are able to suggest a theory of asynchronous development within the closely integrated cell dynamics of anagen follicles.

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(4) Pubertal induction of sweat gland activity

J.Rees and S.Shuster, *Department of Dermatology, University of Newcastle upon Tyne.*

We have studied the well-known observation that men sweat more than women. Sweat was induced by iontophoresis of 2% pilocarpine, and sweat was collected over a 15 min period onto 3 cm filter paper discs inside aluminium capsules stuck to the skin, and weighed as described elsewhere (Johnson & Shuster, 1969). Sweat in eight normal males was found to be about double that in nine normal females ($P < 0{\cdot}005$). By contrast, sweat rates were equal in six normal boys and six normal girls aged 9–10 years, and when their sweat rates were corrected to adult body surface area they were the same as those of normal adult females. Thus the difference in sweat rate between adult men and women is due to an increase in the males at puberty. Since this difference does not occur in adults with life-long hypergonadism, it appears to be due to androgen. Despite this, androgen, both topically and by ID injection, did not increase sweat rate in twelve normal females. An antiandrogen (flutamide) did not decrease sweat rate when applied topically to nine normal males, nor did cyproterone acetate when given systemically to seven males treated for acne although their sebum excretion rate decreased.

We therefore conclude that although the difference in sweat rate between males and females is initiated by androgen at puberty it is not maintained by androgen. Thus the probable mechanism is by androgen-induced gene expression.

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(5) Serum 25-OH vitamin D after photochemotherapy

S.Shuster, L.Chadwick, Celia Moss and Janet M.Marks, *Department of Dermatology, University of Newcastle-upon-Tyne.*

In a preliminary study we found an increase in the blood concentration of 25-hydroxy vitamin D (25-OHD) in patients with psoriasis treated by photochemotherapy with 8-methoxypsoralen (8-MOP) and long-wave UV irradiation (UV-A, 320–400 peak wavelength 365 nm). This observation was surprising because it is believed that vitamin D_3 synthesis from 7-dehydrocholesterol is promoted in the epidermis by UV-B (280–310 nm). The present study was therefore done to confirm the original observations and to see whether the effect was due to the augmentation of UV-A action by 8-MOP or by UV-B produced by the UV-A irradiation unit.

Serum 25-OHD was measured by competitive protein binding, in thirteen patients with discoid psoriasis, at the following times: before treatment with 8-MOP and UV-A (PUVA), 24 h after UV-A alone, 24 h after 8-MOP alone, and after administration of PUVA (24 h after UV-A, 26 h after 8-MOP).

The results confirm our previous observation of an increase in serum 25-OHD after PUVA. There was no significant increase after UV-A alone, or after 8-MOP alone, showing that the effect of PUVA on epidermal vitamin D_3 synthesis is due to the combined action of UV-A and 8-MOP. This also excludes the possibility that the effect of PUVA was due to a direct action (independent of 8-MOP) of adventitious UV-B emitted by the irradiation unit. These findings show that, contrary to current beliefs, either the action spectrum of 8-MOP is not limited to UV-A, or the synthesis of vitamin D_3 in the skin is promoted by UV-A as well as UV-B.

(6) Advanced cutaneous T cell lymphoma (tumeurs d'emblée type): the differential capacity of E cell rosetting, non specific esterase staining and anti-HTLA peroxidase methods for cytological diagnosis

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The diagnosis of cutaneous T cell lymphoma depends on cytological typing but T lymphocyte markers are sequentially lost during *in vitro* transformation.

Sections from the cutaneous nodules of a 71-year-old male with tumeurs d'emblée type cutaneous T cell lymphoma were examined by three commonly used methods for T lymphocyte identification.

(1) Collagenase incubated skin was mechanically disintegrated to release viable cells. The proportion of cells retaining the ability to rosette sheep red cells was estimated.

(2) Cryostat sections of prefixed frozen tissue were stained for the presence of acid-α-naphthyl acetate esterase activity.

(3) Tissue sections were incubated with anti-HTLA serum and peroxidase conjugate, and examined by light and electron microscopy to reveal the proportion of cells retaining the HTLA membrane antigen.

Six percent of the extracted cells were capable of E rosetting. ANAE activity of T pattern was present in only occasional cells. The immunoperoxidase technique demonstrated characteristic positive labelling of the majority of infiltrating cells. Ultrastructural examination confirmed the presence of HTLA membrane antigen on the cell membrane.

The results demonstrate, *in vivo*, the persistence of HTLA membrane antigen in cutaneous lymphoma T cells which have lost their ability for E rosetting and their ANAE activity. They support the *in vitro* findings of sequential loss of cell characteristics and suggest that the demonstration of HTLA may identify T lymphocytes which could otherwise be considered as null cells.

(7) Amyloid P-component in cutaneous amyloidosis: a new diagnostic immunofluorescence technique

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Amyloid P-component (AP), a glycoprotein identical to the normal plasma protein, serum amyloid P-component (SAP), is always present in primary, secondary and endocrine-associated amyloid deposits (Pepys *et al.*, 1977). It is also a normal constituent of vascular basement membrane (Dyck *et al.*, 1980a, b). We report here the demonstration of AP in deposits of primary localized cutaneous amyloidosis.

Biopsies of involved skin were obtained from six patients with macular and five with papular lichen amyloidosus, and from one each with primary localized nodular and myeloma-associated amyloidosis. Biopsies from seven normal subjects and twenty-eight patients with unrelated dermatoses, including four with eczema, seven with lupus erythematosus, four with lichen planus, five with pemphigoid, three with erythropoietic protoporphyria and one with porphyria cutanea tarda served as controls.

Cryostat sections 4 μm were stained by immunofluorescence with fluorescein-labelled $F(ab')_2$ fragments of rabbit anti-human SAP. Immunological specificity of staining was established by inhibition controls in which the fluor was absorbed with pure SAP.

Morphologically distinctive specific staining of papillary deposits with anti-SAP was seen only in amyloidosis. It was clearly distinguishable from perivascular staining in porphyria. Non-specific staining of stratum corneum was observed in all sections. AP was not present at the dermo-epidermal basement membrane zone in normal skin. Specific staining of dermal elastic fibres was observed in all sections. Immunofluorescence with anti-SAP is simple and specific, and may become the procedure of choice in the diagnosis of amyloidosis and its differentiation from other cutaneous hyalinoses.

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(8) Circulating immune complexes, cryoglobulinaemia and serum complement levels in cutaneous vasculitis

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Patients with biopsy proven cutaneous vasculitis were studied for the presence of circulating immune complexes (CICs) using two different radioimmunoassays: Clq binding (Pussell *et al.*, 1978) and monoclonal rheumatoid factor binding (Halla *et al.*, 1978). Sera for cryoglobulin estimation were obtained simultaneously and the level evaluated according to Brouet *et al.* (1974). Factor B conversion and C_3 conversion products (C_3C) were measured using cellulose acetate membrane electrophoresis and immunofixation (Whicher *et al.*, 1980).

Of the eighteen patients studied, five had necrotizing vasculitis associated with purpuric skin lesions, seven had leukocytoclastic vasculitis associated with urticarial skin lesions, and two patients exhibited a non-purpuric papular eruption with histological features of both dermatitis herpetiformis and leukocytoclastic vasculitis. Four patients with recurrent erythema multiforme of unknown aetiology were also included in this study.

Two patients, one with purpuric and one with urticarial vasculitis, were found to have high levels of mixed cryoglobulinaemia with a monoclonal antibody component. Both exhibited an abnormal complement profile characterized by low CH_{50}, low Clq, C_2 and C_4, with normal levels of C_3 and no evidence of C_3 conversion.

Two further patients with urticarial vasculitis were found to have hypocomplementaemia, and this was associated with high levels of Clq binding and raised levels of C_3C during periods of disease activity. Moderately elevated levels of Clq binding were associated with normal complement profiles without evidence of C_3 conversion.

CICs were detected in patients from all groups by both Clq binding and monoclonal rheumatoid factor binding. Although the level of CIC obtained by these two assays did not always correlate in individual cases, no patient was consistently positive by one assay only. Small amounts of cryoglobulin (40–200 μg/ml) were commonly present in patients with CICs, but these patients could not be separated clinically from non-cryoglobulinaemic sera.

These findings support the thesis that the sera of patients with cutaneous vasculitis contain a heterogenous population of CICs, that the type of CICs cannot be used as a method of discriminating between different clinical varieties of cutaneous vasculitis, and that the detection of small amounts of cryoglobulin represents another method of identifying CICs, and does not necessarily correlate with clinical syndromes that are induced or exacerbated by cold exposure. Patients with larger amounts of circulating cryoglobulin (>1 mg/ml) can be separated clinically since cold exposure is an important localizing factor in this variety of complement-mediated cutaneous vasculitis.

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(9) Host-parasite relationships in hyperkeratotic scabies

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The presence of large numbers of parasites in (and on) the skin of patients with hyperkeratotic (Norwegian) scabies allows easy microscopic observation of the host-parasite relationships.

Material for this study was obtained from the skin lesions of three subjects with hyperkeratotic scabies. Unfixed skin specimens from two patients were incubated *in vitro* in the presence of ^{3}H thymidine and after fixation in formaldehyde, sections were processed for histo-radioautography. More than 600 serial sections were studied under the light microscope. Scales were also observed by scanning electron microscopy.

The observation of this material allowed us to define more precisely how the scabies mite induces skin lesions and the dynamics of the host's response to the invader.

At first, mites progress through the layers of the stratum corneum, but tend to reach the living parts of the epidermis. Some microns ahead of the buccal parts of the mite, vesiculation resulting from cell necrosis was observed. Ingestion of the liquefied epidermis creates an empty space where eggs, embedded in an amorphous adhesive material, are laid down on the parakeratotic floor of the newly formed burrow. When larval maturation is achieved, the mites leave the egg shells which remain attached to the parakeratotic floor. Some mites invade the papillomatous epidermis down to the dermo-epidermal interface. In one instance, basement membrane disruption was noted. Extravasated red blood cells are present in burrows.

In the dermis, leucocytes (mainly mononuclear cells) migrate out of elongated and dilated capillaries and exocytosis of those elements is seen predominantly in the vicinity of the mite. This reaction pattern occurs rapidly and could be related to factors inducing the focal process of epidermal necrosis. Spongiosis is also maximal at this level.

Increased ^{3}HT labelling of basal cells and epidermal hyperplasia as well as the increased ^{3}HT labelling index of the dermal mononuclear cells reflect the reactive process of the host in which immunocompetent cells are involved.

(10) Loss of β_2 microglobulin from the surface of cutaneous malignant and premalignant lesions

R.M.MacKie and M.L.Turbitt, *Department of Dermatology, University of Glasgow.*

At present the cell surface changes associated with the transition from benign to premalignant to malignant counterparts are poorly understood. They are, however, essential to our understanding of events leading to metastatic spread of tumour cells.

β_2 Microglobulin is the light polypeptide chain of the human leucocyte antigen (HLA) system and is found on the surface of all normal human cells *in vivo*.

We have studied β_2 microglobulin on the surface cell of twenty-three cutaneous malignancies and twenty-two premalignant or benign skin tumours by the immunoperoxidase immunoenzyme bridge technique. All benign skin tumours (basal cell papillomas, keratoacanthomas) showed β_2 expression on the cell membranes and all malignant tumours (squamous carcinomas, Bowen's disease and basal cell carcinoma and malignant melanoma) showed an absence of surface β_2 microglobulin. Actinic keratoses were used as a model of a premalignant condition and in eight out of ten cases some degree of loss of β_2 microglobulin staining was observed.

It would therefore appear that loss of the β_2 microglobulin cell surface marker correlates well with cell surface changes associated with malignant transformation.

(11) Spontaneous (NK cell) and antibody-dependent (K-cell) lymphocyte cytotoxicity in systemic sclerosis

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Spontaneous (NK cell) and antibody-dependent (K-cell) lymphocyte cytotoxicity were investigated in thirty-nine patients with systemic sclerosis (SS) and compared with that found in fifty-two normal controls. ^{51}Cr labelled Chang liver cells were used as targets in assays utilizing both whole blood (WB) and peripheral blood mononuclear cells (PBM) as effectors. A good correlation between the results of NK and K cell assays was found in both patients and controls using both WB ($r = 0.82$, $P < 0.001$; $r = 0.71$, $P < 0.001$) and PBM ($r = 0.63$, $P < 0.001$; $r = 0.65$, $P < 0.001$) assays respectively.

Patients with SS, who were severely affected by extensive visceral disease, were found to have significant impairment of both NK ($P < 0.005$; $P < 0.05$) and K ($P < 0.001$; $P < 0.05$) cell cytotoxicity using both effector systems, when compared with the normal controls.

These findings could result from either an intrinsic defect in cell-mediated immunity or from the effects of circulating immune complexes or anti-lymphocyte antibodies. They may also provide a possible explanation for the described association of malignancy with systemic sclerosis.

(12) A comparative study of psoriatic and non-psoriatic buccal epithelium

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In psoriatic epidermis there is an increase in epidermal labelling index (LI) and a decrease in the amount of a 70K molecular weight tonofilament protein. Normal buccal epithelium has a higher LI than epidermis and, in the present study, LIs and protein compositions have been determined for psoriatic and non-psoriatic buccal epithelium. This was to establish whether there is a difference between the two groups and if any difference in LI associated with or occurring independently of psoriasis can be correlated with contents of 70K protein.

Fourteen patients were studied—seven with psoriasis (either widespread chronic plaque type or erythrodermic, mean age 48·3 years) and seven without psoriasis (urticaria, basal cell carcinoma or leg ulcer, mean age 59·5 years). Two adjacent 4 mm punch biopsies were taken from clinically normal lateral buccal mucosa under local anaesthesia.

A LI was determined for each patient using standard autoradiographic techniques. No significant difference was found between the psoriatic LI ($29{\cdot}0 \pm 3{\cdot}3\%$) and the non-psoriatic LI ($27{\cdot}0 \pm 6{\cdot}8\%$) ($P > 0{\cdot}01$).

The amounts of 70K protein, determined by SDS-gel electrophoresis, were found to be low and variable in both psoriatics and non-psoriatics and there was no difference between the groups. No correlation was observed between the amount of 70K protein and the LI of individual samples.

The study did not confirm an increased LI previously reported in psoriatic buccal epithelium (Kaidbey & Kurban, 1971). The 70K protein is decreased or absent in both psoriatic and non-psoriatic buccal epithelium and this is not related to the LI or to the presence or absence of psoriasis.

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(13) The pharmokinetics of 8-methoxypsoralen in patients treated with PUVA

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Twenty patients receiving PUVA therapy for treatment of psoriasis were studied to determine the kinetics of 8-methoxypsoralen (8-MOP) in relation to the response of patients to 8-MOP-UV-A therapy.

Patients were divided into three groups on the basis of their response, the three groups being good, moderate or poor responders.

Patients were fasted for 12 hours prior to administration of 8-MOP. The drug was administered orally at a dose of 0·6 mg/kg and blood samples were collected at 0, 0·5, 1, 1·5, 2, 2·5 and 3 h and hourly thereafter to 8 h. Plasma concentrations of 8-MOP were determined by a sensitive and specific high pressure liquid chromatography assay.

The elimination half life of 8-MOP was lowest in the poor responders, but this did not attain statistical significance. The area under the plasma concentration curve was significantly ($P < 0{\cdot}05$) lower in the poor responders than in both the good and moderate responding groups, as assessed by the Mann-Whitney U test. The peak plasma concentration was lowest in the group of poor responders, but this did not attain statistical significance. The time to attain peak plasma concentrations was not significantly different between the groups.

It is apparent from these studies that poor response to PUVA therapy may therefore be associated with differences in 8-MOP kinetics.

(14) MPF potentiates MSH-induced melanogenesis in the mammal

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Melanotrophin potentiating factor (MPF), the C-terminal tetrapeptide of β-lipotrophin (βLPH_{88-91}) potentiates the action of melanocyte-stimulating hormone (MSH) on *Anolis* skin. In the present study we have tested the activity of MPF on MSH-induced melanogenesis in the hair follicle of the hamster.

Hair follicles taken from Siberian hamsters (*Phodopus sungorus*) during their spring moult were cultured for 48 h with either αMSH (dose range 10^{-6} to 10^{-9} mol/l) or MPF (10^{-8} mol/l) or αMSH and MPF together. Melanogenesis was monitored by measuring tyrosinase activity in a radiometric assay and melanin accumulation by a specific fluorimetric method.

αMSH caused a dose-related increase in both tyrosinase activity and melanin production over the range tested, optimal stimulation occurring at 10^{-7} mol/l. MPF had no effect on melanogenesis on its own but the tyrosinase and melanin responses to αMSH, throughout the dose range, were significantly greater ($P < 0{\cdot}01$) in the presence of MPF.

MPF therefore potentiates the action of MSH on melanogenesis in melanocytes as well as melanosome dispersion in melanophores. Although these phenomena involve different intracellular events, it is likely that both types of pigment cell possess similar receptor mechanisms. MPF may therefore effectively lower the levels of MSH peptides required to stimulate pigmentation. The hormonal control of pigmentation may need to be re-assessed in the light of these findings and the possibility that MPF modulates the effects of MSH in other tissues, such as the brain, must also be investigated.

(15) The biosynthesis of human epidermal prekeratin

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The formation of keratin filaments is a prerequisite for normal keratinization, the initial stages of which include the biosynthesis and post-translational modifications of prekeratin polypeptides in living epidermal cells. It is possible to translate messenger RNA (mRNA) from human epidermis in a cell-free system and to isolate the initial gene products by precipitation with antiserum to prekeratin (Bowden *et al.*, 1980). We have now investigated the next stage of keratin formation, the biochemical processing of the initial gene products, by comparing native prekeratin with the products of *in vitro* incubation and cell-free biosynthesis.

Slices of scalp epidermis were incubated with labelled amino acids and processed prekeratin isolated either by immunoprecipitation with anti-prekeratin IgG bound to protein A-sepharose or by isoelectric precipitation (Skerrow, 1977). Native epidermal prekeratin was prepared and was labelled with [^{14}C]-formaldehyde (Rice & Means, 1971). The products of mRNA-directed cell-free translation were isolated by immunoprecipitation and the labelled proteins compared by SDS-PAGE (Bowden & Cunliffe, 1980) and fluorography.

Human scalp epidermis incorporated labelled amino acids into prekeratin, which had similar electrophoretic behaviour to native prekaratin. However, the products of mRNA-directed biosynthesis contained fewer polypeptide chains than prekeratin synthesized by incubation of epidermal tissue. As the translation of mRNA in the reticulocyte lysate system yields essentially unprocessed polypeptide chains, it is possible that the more complex polypeptide chain composition of native and *in vitro* biosynthesized prekeratin is due to post-translational modification. The exact molecular nature of this mechanism is presently under investigation.

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(16) The composition of prekeratin and keratin from psoriatic epidermis

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It is well established that the fibrous protein from stratum corneum (keratin) differs from, and is derived from, that synthesized (prekeratin) in the living epidermis. Plantar stratum corneum (callus) and psoriatic scale keratins are atypical in composition (Baden *et al.*, 1978; Skerrow & Hunter, 1978; Thaler *et al.*, 1978) but there is little data on the corresponding prekeratins. The aim of the present study is to compare keratin and prekeratin from normal and pathological epidermis.

Epidermis from scalp skin, heel skin and various psoriatic lesions was extracted with acid buffer and prekeratin obtained by isoelectric precipitation (Skerrow, 1977). Normal stratum corneum, heel callus and psoriatic scale were extracted with neutral 6M urea containing 2% mercaptoethanol, and dialysed against acid buffer. The keratins were obtained by isoelectric precipitation and compared to prekeratin by high resolution SDS-PAGE on gradient slab gels (Bowden & Cunliffe, 1980).

Prekeratin from normal epidermis was composed of several polypeptides of molecular weight 45,000–70,000. Callus prekeratin contained three additional polypeptides (mol wt 44,000, 50,000 and 65,000). Psoriatic prekeratin was characterized by a lack of larger polypeptides (mol wt. 67,000–70,000) and the presence of two polypeptides (mol wt 44,000 and 50,000) normally restricted to plantar epidermis. Psoriatic scale demonstrated a prekeratin-like composition and unlike normal stratum corneum, involved callus did not markedly differ in composition from involved scale at other sites.

We have demonstrated that prekeratins from plantar and involved psoriatic epidermis differ from each other and from normal prekeratin. These differences may result from changes in keratin gene expression combined with, in psoriatic epidermis, a lack of post-translational modification.

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(17) The effect of tunicamycin on epidermal glycoprotein, glycosaminoglycan and protein synthesis *in vitro*

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Tunicamycin (TM) is an antibiotic which blocks protein glycosylation by inhibiting the synthesis of lipid-linked sugar intermediates, thus preventing the transfer of sugars to asparagine residues in glycoproteins. As part of our continuing study on the structure and function of epidermal cell surface carbohydrates we have examined the effect of TM on the synthesis of epidermal glycoproteins and glycosaminoglycans in organ culture.

TM was a generous gift from Professor G.Tamura, University of Tokyo. It was dissolved in 25 mM NaOH at a concentration of 2 mg/ml. Slices of pig ear skin (1 cm square, 0·2 mm thick) were maintained in organ culture in the presence of D-(^{3}H) glucosamine (10 μCi/ml) and ^{14}C-amino acid mixture (1 μCi/ml) together with increasing concentrations of TM. After 18 h the epidermis was separated from dermis and was solubilized in 8 M urea, 5% sodium dodecyl sulphate at 100°C. The total incorporation of D-(^{3}H) glucosamine into the epidermis was inhibited approximately 40–45% by TM at 1 μg/ml or greater. This effect was specific since the incorporation of ^{14}C-amino acids was unaffected by concentrations of TM up to 5 μg/ml. Polyacrylamide gel electrophoresis of the solubilized epidermis indicated that TM had little effect on the labelling of the glycosaminoglycan peek (mainly hyaluronic acid) with D-(^{3}H)glucosamine. However, the labelling of the faster migrating glycoprotein components was considerably reduced.

Subcellular fractionation showed that the major effect of TM was on the synthesis of particulate rather than soluble glycoproteins. Thus the incorporation of D-(^{3}H) glucosamine into the glycoproteins of the plasma membrane enriched fraction was inhibited 70% by TM at 1 μg/ml. This would suggest that most of the membrane glycoproteins in the epidermis contain asparagine linked oligosaccharides.

Electron microscope examination of TM treated epidermis revealed surprisingly little alteration in cell surface morphology. Desmosomes appeared normal, indicating either that the carbohydrate portions of glycoproteins do not make a significant contribution to these structures or, more probably, that there is little turnover of their glycoprotein components in organ culture. The endoplasmic reticulum was often dilated and in longer term cultures the cytoplasm appeared increasingly vacuolated.

POSTER DISCUSSION. CHAIRMAN—DR R.A.J.EADY

(18) Cuticular scale size and diameter studies of human hair

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Five hairs cut from each of ten sites in twenty male and twenty female volunteers without present or past skin disease were examined by scanning electron microscopy at standard angle and magnification. Three measurements of mean scale number (MSN) and three measurements of diameter were made on each negative projected in a microfische reader. The results reported are based on 6,000 MSN and 6,000 diameter measurements.

Statistical analysis of the MSN results permitted the division of sites into three distinct groups—$P < 0{\cdot}001$:

(a) scalp with the lowest MSN;
(b) eyebrow and eyelash with the highest MSN;
(c) face, axilla, forearm, chest, abdomen and lower leg with an intermediate MSN.

Between males and females a statistical difference in MSN is seen at nine out of ten sites at a lower level of significance ($P < 0{\cdot}05$).

A positive correlation is found between the results and hair growth rates already published (Barman *et al.*, 1969; Saitoh *et al.*, 1969).

The examination of hair diameters confirms the already held view that hairs at androgen dependent sites are wider than hairs on the rest of the body. Significant differences ($P<0.05$) occur between males and females on the face, chest, pubis and lower leg.

A comparison of MSN and diameter in this study shows no overall correlation but neatly throws human terminal hairs into three morphological groups:

(1) Eyelash and eyebrow.
(2) Male face, chest and pubis and female pubis.
(3) All other hairs examined.

From this latter group scalp hair is normally separable by its length.

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(19) Epidermal changes occurring in histamine weals and cold urticaria

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A study was made of the sequential changes in epidermis caused by the influx and dissipation of oedema associated with acute inflammation.

Skin biopsies were taken before and after cold challenge in 10 patients with cold urticaria and after I.D. injection of 50 μg histamine in PBS pH 7·4 into normal skin of ten further subjects.

Light microscopy of 1 μm thick Epon sections showed comparable changes in cold urticaria and histamine weals. Up to 30 min following challenge or injection there was widening of the intercellular space and apparent melanocyte and keratinocyte vacuolation except in appendigeal epidermis. Langerhans cells and stratum corneum were undisturbed. Melanocytes became pendulous, often suspended from the epidermis by a solitary attenuated dendrite. At 4–24 h following challenge or injection, all features returned to normal. Serial measurements of the thickness of the lowermost three cell layers of interfollicular epidermis failed to reveal significant epidermal thickening in either histamine weals or cold urticaria.

Electron microscopy showed that the apparent *intercellular* oedema was chiefly *intracellular*, and located at the periphery of keratinocytes where tonofilament density was reduced. Bleb-like keratinocyte processes impinged upon melanocytes giving rise to the false impression of melanocyte vacuolation. Membrane-bounded vacuoles were, however, present in the perinuclear zone of numerous keratinocytes.

The findings have shown that in acute inflammation there is rapid influx of oedema into epidermis and that it appears to collect chiefly within subcorneal keratinocytes. There was no suggestion of efflux through the stratum corneum.

(20) Is there a relationship between stratum corneum breaking load *in vitro* and intracorneal cohesion *in vivo*?

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Measurement of intracorneal cohesion (ICC) by cohesography characterizes the binding forces within the superficial stratum corneum (SC). In large part it reflects desquamation and abnormalities of this are paralleled by changes found by cohesography. Determinations of elasticity of SC *in vitro* are performed in the horizontal dimension and reflect the physical properties of the entire thickness of this structure. This study relates these two fundamental properties of the SC so as to give increased significance to each of the measurements. Cohesograph readings from the lateral aspects of the thighs were made on normal male volunteer subjects and from individuals with congenital disorders of keratinization causing scaling. Skin was then removed from the same areas and the SC separated by a trypsinization procedure. The thickness of these specimens was measured by surfometer and the breaking load determined by a specially constructed microtensometer under controlled ambient conditions. Width, stressed length and rate of strain were the same for each tested specimen. Linear regression analysis between ICC and *in vitro* breaking load for all subjects investigated gave a correlation coefficient of 0·77 ($P<0{\cdot}02$). As may be expected, when the results of *in vitro* breaking load are 'normalized' (per μm thickness) and plotted against ICC, no clear correlation is observed. The relationship between ICC and *in vitro* breaking load has considerable functional importance and suggests that cell to cell contacts within the SC are similar with regard to cohesive strength in both horizontal and vertical dimensions. This study further validates cohesography as an *in vivo* method for studying SC function.

(21) Prevalence and significance of basal liquefactive change in solar keratoses

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Spontaneous regression is an occasional feature of several neoplastic disorders which, if better understood, might give insight into the immunology of neoplasia. Lichenoid keratoses show basal cell liquefaction similar to that in lichen planus, suggesting immunologically mediated epidermal cell damage. As a first step in determining the factors responsible for this change, we have (a) characterized the prevalence of basal liquefactive degeneration (BLD) in solar keratoses, (b) correlated the other main histomorphological features of solar keratoses with the presence of BLD, and (c) examined solar keratoses for immunoglobulin deposition and sera from patients with keratoses for antibodies to epidermal cells.

Of 212 keratoses, 27·8% showed some BLD, but only 1·4% showed this feature extensively. Histomorphological correlations in ninety-seven keratoses could not identify any one feature which appeared particular to lesions showing BLD. In thirty keratoses examined by the direct immunofluorescence technique, IgM was found in 50% either in discrete round deposits or as a 'ragged band' at the dermoepidermal junction. A similar but weaker deposition of IgA deposits was also found in some lesions. No circulating antibodies were detected in indirect immunofluorescent tests. Histological examination of these thirty lesions could not identify any feature particular to those lesions that demonstrated immunoglobulin deposition. It seems likely, therefore, that the epidermal cell damage is cell mediated, but that the density of the inflammatory infiltrate is not related to the presence of the particular cell population responsible. Work is currently directed to examining the integrity of epidermal cell membranes of lesions showing BLD and to identifying the cell types in the infiltrate.

(22) The influence of psoriasis on hair diameter

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Hairs were cut from equal numbers of psoriatic patients of both sexes from affected and never affected sites until 20 aliquots had been obtained from each of the following sites: scalp, eyebrow, eyelash, face, axilla, forearm, chest, abdomen, pubis and lower leg. Control samples were then obtained from twenty males and twenty females without present or past skin disease, matched for age. Three diameter measurements were then made from negatives of scanning electron micrographs taken from each of five hairs sampled in each aliquot using a constant angle and magnification and projected in a microfische reader at a standard setting; 18,000 measurements were thus obtained for analysis.

Overall results showed a decrease in diameter, ($P<0{\cdot}001$) in hairs taken from affected plaques, compared to unaffected skin and to controls. Individual sites showed a significant decrease in diameter ($P<0{\cdot}05$) in eight out of ten sites for males and in six out of ten sites for females in comparing affected and unaffected skin. In all forty individual comparisons the 'affected' hairs were invariably narrower than the other categories.

Comparing 'unaffected' hairs with controls, ten out of twenty comparisons showed significant narrowing ($P<0{\cdot}05$)—further evidence for the concept that the apparently normal skin of psoriatic patients is not functionally normal.

(23) The influence of topical treatments on corneocyte size in normal skin

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Corneocyte size (as assessed by corneocyte area) is decreased in eczematous dermatoses (Hölze & Plewig, 1977), psoriasis (Goldschmidt, 1979) and the ichthyotic disorders (Nicholls *et al.*, 1978), and treatment of these conditions restores this measurement to normal. We have previously demonstrated that stratum corneum barrier function is at least partially dependent on corneocyte size (Marks *et al.*, 1980) and factors that influence corneocyte size may modulate function. Corticosteroids cause a reduction in keratinocyte size in normal skin, and this study was started by examining the effects of these compounds on mean corneocyte area in the normal skin of the thighs of volunteer subjects. Fluocinolone acetonide ointment 0·025% was rubbed in twice daily to the right thigh and white soft paraffin was rubbed in twice daily to the left (control). Mean corneocyte area (MCA) was determined by a microscope projection technique on smears of corneocytes obtained from either thigh at intervals. MCA decreased on both thighs. To determine what factor was responsible for this apparent corneocyte shrinkage, other experiments were performed in which the following treatments were applied to the right and left thighs respectively:

(1) Fluocinolone acetonide cream 0·025%—rubbing alone.
(2) Hydrocortisone ointment 1·0%—white soft paraffin.
(3) Arachis oil—polythene film occlusion.

The results indicate that ointment applications caused reductions in MCA, whereas other occlusive treatments did not. The cause of this alteration in size is uncertain, but may be related specifically to the white soft paraffin content of the ointments used. The functional significance of this alteration in size should be borne in mind during formulation of topical therapies.

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(24) The permeability of injured and regenerating rat skin to water

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Diseased and injured skin is more permeable than normal skin. An attempt has been made to establish a well characterized 'injured skin model' in order to measure the relative increases in the absorption of the different classes of chemicals through injured and regenerating skin.

In vivo (ventilated chamber) and *in vitro* (diffusion cell) methods were used to measure water permeability. Flank epidermis of anaesthetized rats was injured by four reproducible techniques: the permeability change varied with the technique (factor of increase *in vivo* was between c.64 and c.4 times normal).

Graphs of 'log increased water loss' versus time were linear (exponential decay) for the early phase (major portion) of barrier regeneration. The rate of regeneration was characterized for the types of injury by the half-times for change in permeability. Similar increases in water loss through injured, i.e. tape-stripped, human epidermis were followed by the same pattern of, but slower barrier regeneration.

The permeability of reproducibly injured rat skin can be related to the permeability of abnormal human skin by water loss measurements.

(25) A comparison of techniques for the measurement of transepidermal water loss in abnormal skin

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The magnitude of transepidermal water loss (TEWL) relates to the integrity of the skin's permeability barrier, the impairment of which has direct relevance to dermal toxicity evaluations. Two methods for measuring TEWL, as one parameter of barrier status, have been compared. Using a ventilated chamber (VC) and a Servomed Evaporimeter EP1 (EP), TEWL has been monitored from normal, physically (tape-stripped) and chemically (n-hexadecane) treated skin of anaesthetized rats.

TEWL readings in normal skin were approximately 4 (EP) and 8 (VC) $g/m^2/h$. Immediately following tape-stripping of the skin maximal TEWL was registered at 75 (EP) and 190 (VC) $g/m^2/h$. As TEWL values declined similar readings were achieved with both types of apparatus from 60 h and until normal levels were regained (after 190 h). The delayed maximum TEWL values observed following n-hexadecane application, over a range of dose levels, were identical using the VC and the EP (30 $g/m^2/h$ at 52 h at the highest dose applied).

It is concluded that under the environmentally controlled conditions employed the EP apparatus achieves comparable sensitivity and reproducibility of TEWL measurements to that of a VC. This relationship however is not consistent in instances of high (>75 $g/m^2/h$) TEWL. Within defined upper limits, an EP, which, in contrast to a VC, is portable and simple to use, may have potential in the functional assessment of the skin's barrier in toxicity studies.

(26) Fibroblasts cultured from psoriatic skin: normal or abnormal?

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Preliminary findings are presented from a comparison of six strains of fibroblasts from the lesional forearm skin of psoriatic in-patients, three strains from normal-appearing skin adjacent to lesions, and six strains from forearm skin of normal subjects. Fibroblasts were grown and maintained by techniques previousley described and studied at passages 3–8.

Growth rate (percentage increase in cell number between days 3 and 6 of culture) of some psoriasis strains was above the normal range with strains from lesional and adjacent skin from the same patient giving similar values, but the mean values [controls 103±16 (s.e.m.), lesional 155±33, adjacent 182±61] did not differ significantly. Rates of glycosaminoglycans (GAG) secretion were slightly higher in psoriatic strains. The rates of growth and GAG secretion were inversely related in the pooled psoriatic strains ($r = 0{\cdot}79$; $n = 9$; $P < 0{\cdot}001$); although data from control fibroblasts look different, additional strains need to be studied to define a significant regression. Qualitative electrophoretic study of GAG produced by the fibroblasts indicates that the bulk is hyaluronic acid with only small amounts of sulphated molecules in all the strains studied irrespective of origin. Urine from the psoriasis patients contained about twice as much GAG as that from a control group (psoriasis 4·87±0·47 mg/24 h; controls 2·47±0·25; $n = 8$; $P > 0{\cdot}001$). Psoriatic fibroblasts reacted quickly to withdrawal of fetal calf serum from the culture medium by rounding up and detaching from the plastic substrate. Control fibroblasts were not affected.

These results are compatible with the hypothesis that the metabolism of the dermis, and perhaps of other connective tissue, is disturbed in psoriasis. Further work is in progress.

(27) Inhibition of cell-mediated immunity in the guinea-pig by PUVA

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The effect of a 14-day course of PUVA treatment on cell-mediated immunity in the albino guinea-pig has been examined. 8-Methoxypsoralen (0·75 mg in an alcoholic vehicle) was injected intraperitoneally. Long wave ultraviolet radiation (UV-A) was delivered to the depilated right flank. In ovalbumin-sensitized (OA) guinea-pigs, 8-MOP ($n = 24$ animals) and to a greater extent 8-MOP plus UV-A ($n = 22$) significantly decreased the cell-mediated immune reactions (erythema, skin thickness and cellular infiltrate) to intradermal challenge with OA on the unirradiated left flank as compared to untreated controls ($n = 24$), to vehicle-injected controls ($n = 6$), to saline-injected controls ($n = 6$) and to controls treated only with UV-A ($n = 18$). Cutaneous reactions to intradermal injection of turpentine ($n = 24$) on the unirradiated left flank were not affected by 8-MOP and UV-A either singly or in combination. Treatment with 8-MOP plus UV-A reduced the cutaneous reactions to topical dinitrofluorobenzene on the unirradiated side in DNFB sensitized guinea-pigs ($n = 24$). These results indicate that PUVA treatment produces inhibition of cell-mediated immunity. This effect may play a role in the therapeutic effect of PUVA and in its potential to promote skin cancer.

(28) Isolation and polypeptide chain composition of keratinocyte subpopulations from human epidermis

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The observation that trypsinization at 4°C induces a high-level split within the living layers of human epidermis has formed the basis of a method for isolating keratinocytes at different stages of differentiation.

After trypsinization epidermis was peeled from dermis and shaken to separate the tissue along the intraepidermal split. Large clumps of basal and lower spinous cells (B + S) were obtained. Sheets of epidermis from above the split consisted of stratum corneum with the uppermost 4–6 layers of living keratinocytes comprising all the granular cells and the uppermost 1–2 layers of spinous cells. Most of these living cells were detached, without further trypsinization, by stirring (F1) and a further fraction (F2) from the uppermost granular layer was detached by the use of more powerful shearing forces. The highly differentiated cells thus obtained were characterized by their unusual ability to remain flattened in suspension after trypsinization. Tryphan blue exclusion of the various keratinocyte fractions ranged from 75 to 95% and the cells appeared ultrastructurally undamaged.

Analysis of the cell populations by SDS-polyacrylamide gel electrophoresis demonstrated the changes in content of the 63K and 55K tonofilament chains which are known from other studies to occur at some stage in the keratinization process. The present data allow a more precise identification of the epidermal strata in which these changes take place. They demonstrate that the modifications to the tonofilament protein and cell envelope do not occur precipitately at the living epidermis-stratum corneum interface but are already in progress in the spinous and granular layers.

(29) Changes in levels of arachidonic acid and prostaglandins D_2, E_2, F_2 and 6-oxo-PGF$_{1\alpha}$ in human skin within 48 hours following ultraviolet B irradiation

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Increased amounts of substances resembling arachidonic acid (AA), prostaglandin E_2 (PGE$_2$) and prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$) in human skin exudate 6 and 24 h after ultraviolet B (UV–B = 290–320 nm) irradiation have previously been demonstrated, but these concentrations fell to control values at 48 h though the erythema was still maximal (Black *et al.*, 1978a). The rise at 24 h was confirmed by combined gas chromatography-mass spectrometry (GC-MS) (Black *et al.*, 1978b). Intradermal injections of PGD$_2$ produce a long-lasting erythema, and intravenous injections of prostacyclin (PGI$_2$), the parent compound of its stable metabolite 6-oxo-PGF$_{1\alpha}$, produce erythema and vasodilation. In order to study the relationship between UV-B erythema and concentrations of arachidonic acid and its metabolites, levels of AA, PGE$_2$, PGF$_{2\alpha}$, prostaglandin D_2 (PGD$_2$) and 6-oxo-PGF$_{1\alpha}$ in exudates from normal skin and irradiated skin were measured at 10 min, 2, 6, 18, 24 and 48 h after UV-B irradiation. Using Westinghouse sun tubes which have a major emission in the UV-B range, fifty-eight subjects were irradiated with three minimal erythema doses of UV-B on their lower abdominal skin. The erythema was assessed visually, and skin exudate obtained from control and irradiated skin using a suction blister technique. The exudate was equilibrated with deuterated internal standards, acidified and extracted. After preparative thin layer chromatography, zones corresponding to the authentic compounds were eluted with methanol and the dried residues were derivatized for GC-MS estimation. Erythema was barely visible at 2 h, of moderate degree at 6 h, and maximally deep red at 24 and 48 h. AA levels were elevated at 2, 6, 18 and 24 h, falling by 48 h to near control levels. PGE_2 showed a progressive significant elevation from 2 to 24 h, but fell to near normal concentrations at 48 h. $PGF_{2\alpha}$ concentration was raised at 24 h and PGD_2 at 6, 18 and 24 h. Levels of 6-oxo-$PGF_{1\alpha}$ were raised maximally at 6 h, still raised at 24 h, but returned to normal at 48 h. The present findings indicate the UV-B irradiation activates arachidonic acid metabolism, with increased production of the prostaglandins measured. In the first 24 h of the UV-B reaction, there is a relationship between the concentrations of some prostaglandins and the intensity of UV-B erythema. Prostacyclin (PGI_2) may be important in the earlier stages of the erythemal reaction.

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(30) Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol UV-B

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A previous observation of impairment of T lymphocyte responsiveness after photochemotherapy with 8-MOP and long wave UV-A (PUVA) led to a study of the effects of PUVA on cell mediated immunity.

Contact hypersensitivity to DNCB was measured by an improved (quantitative) method, in eighty-five patients with psoriasis and twenty-five healthy controls. Patients were sensitized with 500 μg DNCB, the lowest dose found to sensitize all healthy subjects. Responses to epicutaneous challenge with a series of concentrations of DNCB were measured as volumes calculated from diameter and thickness and the various groups were compared by the differences in the log dose-response curves. Patients with untreated psoriasis were less responsive than healthy controls ($P < 0{\cdot}01$); responses were less still in patient's treated with dithranol/UV-B/tar and they were least of all in patients treated with photochemotherapy with 8-methoxypsoralen and UV-A (PUVA), particularly in those who pigmented least. Sensitization and challenge at different stages of treatment showed that both induction and elicitation of sensitization were impaired by PUVA. These changes may be related to alterations in DNCB metabolism, or to damage to Langerhans cells or circulating T lymphocytes. The clinical significance of impaired contact hypersensitivity in psoriasis and during treatment is not clear. Since dithranol/UV-B/tar has proved safe over many years of use, it seems unlikely that the slightly greater impairment of immunological reactivity after PUVA treatment will prove to be of pathological importance.

(31) Mechanism of vasodilatation due to substance P in humans

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Substance P is a potent vasodilator (Hallberg & Pernow, 1975) and has been shown to occur, and to increase blood flow, in human skin and muscle (Löfström, Pernow & Wahren, 1965) following arterial infusion, although whether its action is direct, acting on vascular smooth muscle, or indirect via the release of another substance is unclear.

In this study, indirect methods were used to assess the mechanism by which Substance P causes cutaneous inflammation in humans. Five subjects received i.d. injections of increasing doses (5–405 nmol) of Substance P at pH 7·2 and the responses were compared with those due to histamine (0·08–1·6 nmol), compound 48/80 (100 ng) and isotonic phosphate buffered saline. The following parameters were assessed: area of erythema at 30 s and 5 min as measured by planimetry, weal (mean of two perpendicular diameters) and subjective sensations.

Both histamine and Substance P produced sigmoid dose-response curves, Substance P being more potent than histamine. Tachyphylaxis was demonstrated by standard methods. Substance P pretreatment induced tachyphylaxis to subsequent injection with Substance P or histamine; also histamine induced tachyphylaxis to Substance P. Saline control was without effect. Erythema following Substance P i.d. was not blocked by a constricting band, indicating a neurogenic mechanism.

Inhibitor studies were performed following oral pretreatment with diphenhydramine, cimetidine, indomethacin and doxantrazole prior to injections. Diphenhydramine inhibited erythema and wealing to histamine and Substance P and dose-response curves for weals were also shifted to the right by doxantrazole. Other drugs produced no significant change in the responses. These results support a possible role for Substance P in cutaneous inflammation acting either directly or via histamine release from dermal mast cells.

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(32) Metabolism of androgens by rat skin *in vivo*

VALERIE A.RANDALL AND F.J.EBLING, *Department of Zoology, University of Sheffield, Sheffield.*

It is currently assumed that the skin resembles the rat prostate, in that its response to androgens requires the formation of 5α-dihydrotestosterone (DHT). In view of its relevance to androgen-dependent skin disorders, we have investigated the uptake and metabolism of testosterone *in vivo* in rat skin and other tissues.

Littermate groups of male rats, castrated 24 h earlier, were injected with radioactively-labelled testosterone and killed at various times. The components of the tissue extracts were separated, after the addition of marker steroids, by partition and development in several chromatographic systems: steroid identification was confirmed by derivative formation and recrystallization to constant $^{3}H:^{14}C$ ratio.

The skin resembled the classical androgen targets in the uptake and retention of both total and non-conjugated radioactivity, but the proportions of the various steroids present were markedly different. Little testosterone was recovered from the ventral prostate where DHT was constantly predominant. In the skin, however, testosterone was always the major free steroid identified and androstenedione, DHT, 5α-androstane-3α, 17β-diol, androsterone, and 5α-androstane-3β, 17β-diol were present only in much smaller quantities, even after 5 hours. The plasma radioactivity mainly consisted of conjugated or very polar steroids, but testosterone and 5α-androstane-3α, 17β-diol were detected at higher concentrations than the other steroids found in the skin.

These results suggest that DHT may not be so important in the response of the skin to androgens as it is in the prostate and that other steroids, most notably testosterone itself, may be involved.

(33) The influence of topical *vs* intravenous administration on the effects of two glucocorticosteroids on DNA synthesis in different tissues of the mouse

R.C.Marshall, M.Burrows, L.G.Brookes and A.du Vivier, *I.P.R. & D.Division, Upjohn Limited, Fleming Way, Crawley, West Sussex.*

Two topical corticosteroids were studied to determine if the difference in their reported systemic effects was due to a difference in percutaneous absorption *per se* or to a difference in intrinsic molecular activity. The compounds, clobetasol propionate and clobetasone butyrate, were administered to hairless mice and DNA synthesis was determined in the epidermis, thymus and spleen by use of ^{3}H-thymidine. DNA was extracted from tissue lysates by hydroxylapatite chromatography and its specific activity measured by UV-spectrophotometry and scintillation counting. Equivalent doses of the steroids were applied topically or injected intravenously in appropriate vehicles.

Following topical application, both compounds showed significant local activity throughout the range of doses tested (20 ng–20 μg). However, whereas 20 ng clobetasol propionate (20 μl; 0·0001%) also elicited significant effects in the distal (untreated) epidermis and thymus, greater than 2 μg clobetasone butyrate (20 μl; 0·01%) were required to elicit similar effects in these tissues. Following intravenous administration, only clobetasol propionate showed significant activity and only then in the epidermis and thymus: 20 μg clobetasone butyrate (200 μl; 0·01%) elicited slight, non-significant effects in the epidermis but not the thymus or spleen. The effects following intravenous administration of these corticosteroid esters were generally lower than those following topical application.

In conclusion, these results suggest that the difference in systemic effects between these two topical corticosteroids is not due to a difference in percutaneous absorption but to a difference in intrinsic molecular activities. This finding is equally valid for effects measured in the epidermis and thymus and, to a lesser extent, the spleen.

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Correspondence

Psoriasis and adnexal structures

SIR, Stankler & Ewen (1981) have highlighted the tendency for psoriasis to affect adnexal structures, sometimes as a major feature. They do not note whether their affected individuals had scalp involvement; certainly the clinical and histological changes were very similar to the scalp parakeratotic comedones described by Kligman (1957). The latter scalp changes are typically associated with peripilar keratin casts (Dawber, 1979) but such casts are not seen on vellus or short, fine terminal hairs. Follicular lesions are not uncommon in psoriasis vulgaris.

Less frequently publicised are the effects of psoriasis on the epidermal eccrine duct unit. In erythrodermic psoriasis, intra-epidermal duct blockage is well recognized (Feibleman, 1971). Repeated Sellotape stripping of the stratum corneum from established anhidrotic plaques of common psoriasis allows some sweating to occur (Johnson & Shuster, 1969), and the halo of hypohidrosis (Suskind, 1954) found on clinically normal skin surrounding all psoriatic plaques can be abolished by Sellotape stripping. Using a skin replicating method described previously, and scanning electron microscopy (Johnson, Dawber & Shuster, 1970), the pathological basis for this change is seen to be superficial sweat pore occlusion (Figs 1–3). We have recently extended this study to examine superficial sweat pore morphology in the 'normal' skin of psoriatic subjects. Seven patients have so far been studied. All had active psoriasis vulgaris. A single replica was taken from normal interscapular skin of each subject. The initial scanning electron microscopic studies have shown that approximately one in seventy sweat pores have the morphological changes of the type previously described in perilesional skin. These changes do not occur in normal subjects (Johnson *et al.*, 1970) and were not seen in the replicas of normal skin examined during the course of other studies (Wilkinson, Marsden & Dawber, 1977; Marsden, Fleming & Dawber, 1979). It has not so far been possible to quantitate these findings more accurately since it is known that only a minority of the pores evident from the starch-iodide or orthophthalaldehyde techniques can be seen by the micropathological method used here (Fig. 1).

These initial studies show that the superficial part of the epidermal sweat duct unit is affected by the psoriatic process. Further studies are in progress to define the changes in the various types of psoriasis during the clinically active quiescent phases.

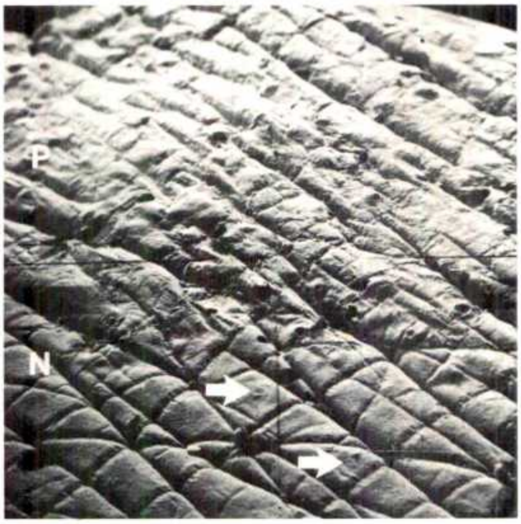

FIGURE 1. Edge of psoriatic plaque showing clinically affected area (P) and normal area (N). Sweat pores (arrowed) are just visible at this magnification (SEM, × 17).

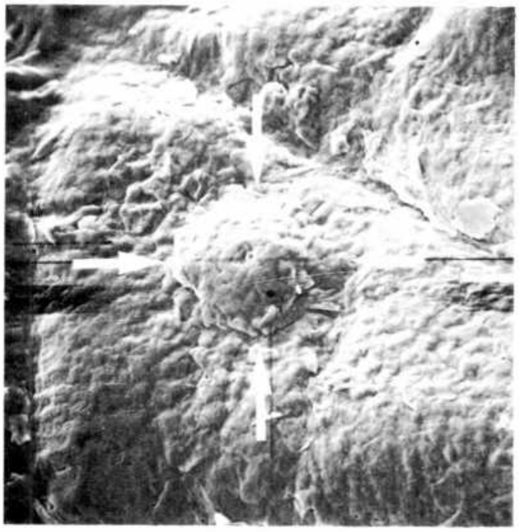

FIGURE 2. An abnormal sweat pore (within arrows) from 'normal' skin adjacent to a psoriatic plaque (SEM, × 280).

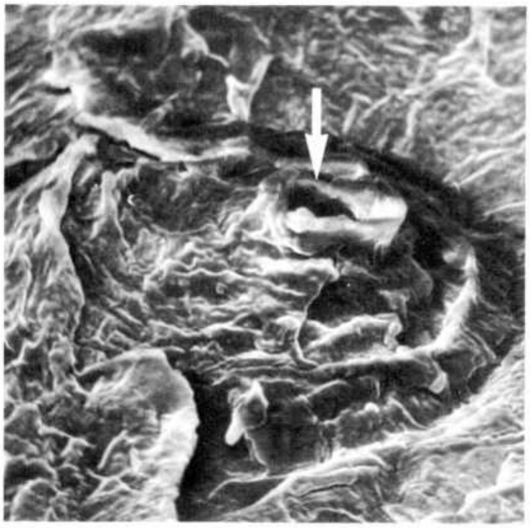

FIGURE 3. A sweat pore opening (arrow) seen in the same area as Fig. 2 after Sellotape stripping (SEM, × 700).

Psoriatic subjects not infrequently get micro-pitting of the palms and soles similar to the pits of Darier's disease (Wilkinson *et al.*, 1977), but considerably smaller than arsenical lesions. We have not so far studied these pits, but it seems quite possible that they are due to parakeratotic material lost from sweat pore openings, analogous to pit formation in nails. It is interesting that the pustules of generalized pustular psoriasis and the microabscesses seen in common psoriatic plaques have been localised to the epidermal sweat duct unit (Neumann & Hart, 1974); perhaps the relationship between the constituents of eccrine sweat and the epidermis deserves a closer look with regard to the early pathogenesis of psoriasis?

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Monosymptomatic hypochondriacal psychosis

SIR, I am interested in studying patients who present with a false conviction of disease or abnormality in a single part of the body, or a single organ system, e.g. 'parasitophobia', 'venereophobia', etc.

I am currently engaged in a data-collection exercise which involves the responsible physician in completing a questionnaire containing items related to the personal and family history of such patients, the specific nature of their complaint and its evolution and their therapeutic history. May I request any of your readers with such patients who would be willing to assist me in this research to contact me with a view to obtaining further information about this project.

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TERENCE M. REILLY

British Journal of Dermatology (1981) **105**, 360.

Book Reviews

Photochemical and Photobiological Reviews, Volume 6. Edited by K.C.SMITH (1981), New York: Plenum Press. Pp. 203. Price £29.50.

Plenum Press and their Editor are to be congratulated on having produced a new volume in this series annually for the last 6 years. This one, unlike most of the earlier ones, has no direct relevance to medicine except for a couple of pages on 'photomedicine' in the chapter dealing with the photoactivation of enzymes, and the two topics there noted are curiosities (the Gunn rat hepatic enzymes and polyamine biosynthesis and skin cancer). Nevertheless this reviewer found something of interest in nearly every chapter.

For those interested in food and drink, there is Chapter 2, by John Spikes, on the photodegradation of foods and beverages. Cooking fats, butter, milk, fresh meat and especially prepared meats such as sausages or ham, are all spoiled to some degree by light. Meat discolouration seems to be due to the formation of metmyoglobin amongst other things. Even hard liquors such as gin, vodka and whiskey are not immune to a light change. Beer is particularly susceptible to what, curiously, is called 'sunburn' offtaste; the photosensitizer is thought to be a flavin or porphyrin; hence the common use of dark brown glass in beer bottles. With wines again it is well known that UVR and light can produce offtastes and turbidity. Hence once more dark bottles. But it has been claimed that certain Spanish reds are improved by illumination and this reviewer had been aware that colleagues in a well known University Department of Dermatology, not a couple of hundred kilometres from the Rioja, had been enjoying studies in wine ageing by UVR.

Daniel Hug's Chapter 3 on photoactivation of enzymes, an updating of his previous review in this series in 1978, covers almost the whole area of photobiology; a difficult task. It is certainly a most interesting and constructive chapter. However he has arranged his material in terms of topics rather than enzyme types or mechanisms, and so the text tends to jerk between somewhat disparate items. Hug's review starts by making the point that enzymatic photoactivation is an example of biological amplification; if a single photon can 'switch on' an enzyme the latter can make thousands of molecules per minute. The chapter ranges from the pineal gland to photosynthetic bacteria, from the ER enzyme to the photoresponses of the phycomoses fungus, from phytochrome to the vertebrate eye, from artificially photoactivated enzymes to certain mysteries, controversies and many other matters. There clearly has been an explosion of work in the field of late.

Chapter 4 is by one of the doyens of photobiology, Dr Arthur Giese. His chapter is on the photobiology of the ciliate protozoan *Blepharisma*. This organism illustrates well some topics in classical photobiology, viz. photodynamic action, photoreactivation, action spectra and the problems in identification of a photosensitizer; the chapter is beautifully written by a noted teacher and scholar.

Chapter 5 by Wolken and Mogus is on the interesting field of extraocular photoreception. In relatively higher vertebrates extraocular photoreception may involve the pineal gland or other brain structures nearby and occasionally a fair amount may be known of mechanisms. But in many lower organisms the photoreceptors lie in the skin itself, perhaps as haem- or caroteinoid-bound proteins.

Some specialists reading through Vol. 6 may think some of the chapters a little superficial, but I think the authors' mandate was generally to cover the chosen fields in an introductory way for the non-specialist. Some of the writers have provided very useful reviews of knowledge thinly scattered over a wide range, in literature difficult to cull. Hug, for instance, offers us about 240 references.

As usual one cannot tell Plenum Press anything about book production in their 'Photochemical and Photobiological Reviews' series; it is superb. The text is in smooth English, and seem to be misprint-free, the diagrams are clear and the single electron micrograph is well produced.

I hope this series continues for many years to come.

I.A.MAGNUS

Epidermal Keratinocyte Differentiation and Fibrillogenesis. Frontiers of Matrix Biology, Vol. 9. Edited by M.Pruniéras (1981) Basle: Karger. Pp. 192, 98 Figs. Price US$72.

This volume of just under 200 pages consists of 10 chapters on keratinocyte differentiation and fibrillogenesis. In general terms the criticism of this work is that with at least one exception it fails to give a balanced view of the subject considered. Although all the material presented is worthy of publication, much of it is more suited to specialized journals.

Even the titles of the chapters imply that there are no possible alternative mechanisms. For example, 'Keratohyaline Synthesis' suggests that there is universal acceptance that keratohyaline granules are actively synthesized (see below). No consideration is given to the possibility that they may be coacervates of hydrolysed cytoplasmic materials.

The first chapter written by the editor and two of his colleagues is complex. It draws attention to the importance of the dermis in epidermal differentiation but concludes that 'tissue differentiation still appears to be largely uncontrolled at the present time'. This the reviewer takes to mean that at the present time the experimental findings have failed to provide evidence of such control. There is no consideration of the possible role of dermal cellular influences in this context.

The second chapter, 'New Markers for Keratinocyte Differentiation', is exciting but unsuitable for a book. It describes in complicated detail the generation of skin antibodies in patients showing the graft versus host reaction following marrow transplants. In this bizarre situation three types were recognized, the pemphigus type, the pemphigoid type, and the keratinocyte cytoplasmic antibody (KCA). The latter showed differing reaction patterns which the authors thought might be demonstrating subpopulations of keratinocytes in differing stages of differentiation. The fact that the Hassall's corpuscles in the thymus also gave positive results with their material is taken to substantiate this contention.

The third chapter entitled 'Biochemical Criteria for Keratinization' deals mainly with sophisticated immunofluorescent techniques. This again is a complicated text which, while going into great detail in some parts, is ambiguous in others. For example, they make the statement that 'under our conditions we found good correlation between the degree of keratinization *in vitro* (observed histologically and histochemically) and the formation of disulphide links'. They do not tell us how they assess the degree of keratinization either histochemically or histologically: the reviewer would like to know of reliable quantitative techniques, and the same applies to the evaluation of disulphide links.

Chapter 4 by G.M.Grey is the exception: it gives balanced views concerning the keratinization of the plasma membrane. This chapter is well worth reading.

There are two chapters on keratohyaline synthesis and synthesis of histidine-rich proteins. Both are worth reading, but in the context that virtually all the material used in their reported experiments was new-born mouse or rat epidermis. Prior to birth the skin is in an aquatic environment which then changes to dry terrestrial surroundings. At this time the skin is without hairs. Thus, during the first week or so of life important alterations have to take place to cope with this sudden environmental change.

The epidermis begins to form keratin, and hair follicles develop. During this period the epidermis has a different histological appearance from that seen at any other time. The epidermis is thicker than in the adult, and keratohyalin granules are present almost throughout the entire thickness of the epidermis. After about 8 days the usual thin rodent epidermis with a single layer of granular cells becomes established. This newborn epidermis was originally used some years ago by Matoltsy to study keratohyalin granules, presumably because so many are present in this epidermis. The same material was used by the San Francisco School for the investigation of the uptake of radioactive amino-acids into keratohyalin, probably for the same reason. The findings are doubtless accurate, but it is probable that they are only applicable to this very short period of neonatal life. It would be surprising if, in this rapidly changing epidermis, synthetic processes were not taking place in the cells of the lower epidermis which at the same time contained keratohyalin granules in considerable quantities. In adult skin the metabolic activity of the cells next to the stratum corneum is considerably reduced, if not completely absent. Thus it is uncertain whether the findings with this unique epidermis can be accepted as typical of all adult mammalian epidermis.

The remaining chapters are concerned with basement membrane formation, microfilaments of the human epidermal-dermal junction, and type IV collagen. A word of warning concerning fibrinogenesis is the possibility of artificial cross linking due to fixation of tissues for electron and light microscopy.

In conclusion, this book is of value, but in the reviewer's opinion, it should be read with the above reservations in mind.

A.JARRETT

The Skin of Vertebrates. Edited by R.I.C.SPEARMAN and P.A.RILEY (1980) London: Academic Press. Pp. 322+illustrations. Price £42.

Everyone who serves on Appointment Committees know that references are usually middling to good. And so are book reviews even though, like the interviewees, books are good, bad or indifferent. That means you must pay much more attention to a bad review than good one. Why has this reviewer broken with the blandness of tradition? Is it misanthropy or because the bad vibes were so strong? In the case of this book I wish to leave you in no doubt: the book is so awful that in these days of enlightened midwifery, editorial amniocentesis should have had it shredded *in utero*

The text is derived from papers given at a skin symposium jointly sponsored by the Linnean Society and the BAD, the purpose of which was to bring together discrete items of knowledge both for dissemination and in the hope of those felicitous indiscretions known as cross-fertilization. Dissemination of knowledge? Observers of trade exhibitions know that the quantity of things people take away with them depends on the innate capacity they bring with them (even though carrier bags are now given in a contemporary spirit of egalitarianism). So for those who read books for tit-bits of knowledge that's fine. After all, collecting knowledge is as good a hobby as collecting antiques and can be shown off as easily (e.g. 'mucus secreted by *Ophicephalus* is said to provide extra strength to mortar and was used for the construction of domes and arches in many of the old churches in Travancore-Tochin', p. 5). But if you read the book to increase your understanding, then don't bother.

And as for cross-fertilization, the only interest I could get out of the accounts of some of the vertebrates was by imagining the bizarre technicalities of their mating. Most authors were content to stake out the limits of their own subject and to present the well-worn patch within it much as we have read it many times elsewhere and the few attempts to cross boundaries were disastrous. What for example is one to think of Singer's apparent belief in the regrowth of human digits and limbs which 'has gone unrecognized by medical doctors although reported in the middle of the last century and perhaps before then'. In support he quotes Sir James Simpson (1856) who described regrowth of limbs *in utero*, and repeated regrowth of a super-numerary thumb after two excisions of it taking 'the ball of it fairly out of the socket'. I read the section three times, each time with increasing incredulity (a) that it was written, (b) that the editors allowed it to stand.

Nonsense abounds by the pageful. It varies from the downright trite, e.g. 'androgenic tumors in women promote beard development (Johnson, 1977)' to the untrue, as when Spearman says 'as in all modulations the changes induced are reversible and in adult male castrates the facial hair reverts to vellus type'. Oh my god, to be thus unmanned by men of science! Apart from the factual error a most interesting aspect of hormonal action is what is now understood about its irreversibility. This datedness of approach appears throughout, e.g. the primitive analysis of sebokeratinocytes, the breathlessly stale use of histochemistry and the sections on Langerhans' cells in which most authors seem to have been caught with their dendrites down. There's a sense too of deja cru about Edna Lawrence's now inevitable repeat of the chalone catechism: I believe in the true Bullough, that auto-regulation occurs through tissue specific and species non-specific inhibitors of tissue growth, contrary evidence notwithstanding. A good example of contrary and inconsistent evidence which is totally ignored (p. 20 and p. 147) concerns the chalone which Bullough (W.S.) found in a cod skin piece in 1967 and has been quoted repeatedly since (p. 142) even though the mitotic burst on which the chalone edifice is built does not occur in fish where, by a supreme irony of personal nomenclature, Bullock (A.M.) showed that wounds heal by cell migration (p. 20). Finally and deplorably there is Lawrence's wild assertion that 'epidermal chalones have been isolated . . . in nearly pure form . . . and are probably glycoproteins or polypeptides'.

One cannot but have sympathy for those like Breathnach, Fraser, Iggo, Sengel, the groups working on plumage, coat colour and recombinants, and others who have contributed so much to their fields, whose articles stand out as belonging to another book.

A desperate disparity is a feature of conference books and like any other book of the show they are usually trite. Enno Christophers had the right answer to the invitation to write up his paper: his text on 'Structural Aspects of Mammalian Epidermal Growth' is less than half a page! Had the other contributors done the same their sections would have been as good and then of course we wouldn't have had the book.

SAM SHUSTER

Dermatological Nursing and Therapy. R.H.SEVILLE and E.MARTIN (1981). Oxford: Blackwell Scientific Publications Limited. Pp. 226. Price £7.50.

This book, as the title suggests, has been written primarily as a nursing text, but it also has a lot to offer the medical student or doctor who is not too well versed in dermatology and its treatment.

The authors believe strongly that stress and psychological factors are of great importance in the aetiology of a variety of skin diseases, and have illustrated their thesis by including 'pen pictures' of actual cases. Not all of these mini-case histories are altogether convincing without further information and in some, such as that of a woman whose diffuse alopecia was attributed to her husband's impotence, one feels that they may be overemphasizing this point. For some of us, however, there is a perhaps a tendency to underestimate the effect of the psyche on skin disease, and this book will at least make us re-think our own personal views on this association.

As would be expected, the section on the treatment of psoriasis is particularly good, with many useful snippets of therapeutic wisdom and could, I suspect, be read with benefit by most Consultant Dermatologists.

One of the strengths of the book is that the authors clearly speak from their own experience, and helpful therapeutic comments and advice are to be found on a variety of subjects.

A number of statements in the book are certainly open to challenge. Many Dermatologists would not agree that seasonal pompholyx is 'thought to be due to inhaled allergens such as moulds', or that in seborrhoeic eczema, overgrowth of normal commensal organisms causes a sensitizing dermatitis.

As a personalized account of the treatment of common skin diseases, I enjoyed reading this book but suspect that it may have difficulty in finding a satisfactory niche. It probably has rather too much information and is over-priced for the student nurse who is not working on a skin ward, and is rather unbalanced for the junior doctor. In spite of this, it contains a lot of useful and practical advice and is worth reading, if only to make us think more about the importance of stress in skin disease, and for the little gems of practical advice that abound, particularly in the chapters on the local treatment of psoriasis.

R.D.G.PEACHEY

British Journal of Dermatology (1981) **105**, 364.

News and Notices

University of Cambridge

The annual Introductory Course on the Biology of the Skin will be held in Fitzwilliam College Cambridge, 8–11 December 1981.

The next 5-yearly course, Progress in the Biological Sciences in Relation to Dermatology, No. 6, will be held during 26–30 September 1983.

Further information may be obtained from The Postgraduate Medical Centre, Clinical School Building, Addenbrooke's Hospital, Cambridge CB2 2QQ.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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The British Journal of Dermatology is owned by and is the official organ of the British Association of Dermatologists. It is also the official organ of the Nederlandse Vereniging van Dermatologie en Venereologie (Netherlands Association for Dermatology and Venereology).

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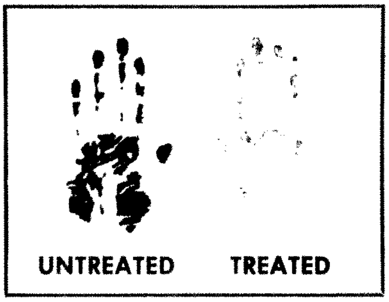

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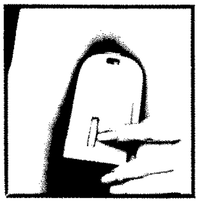

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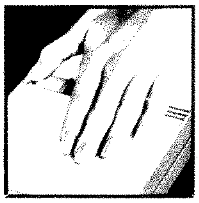

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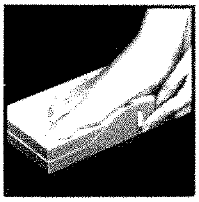

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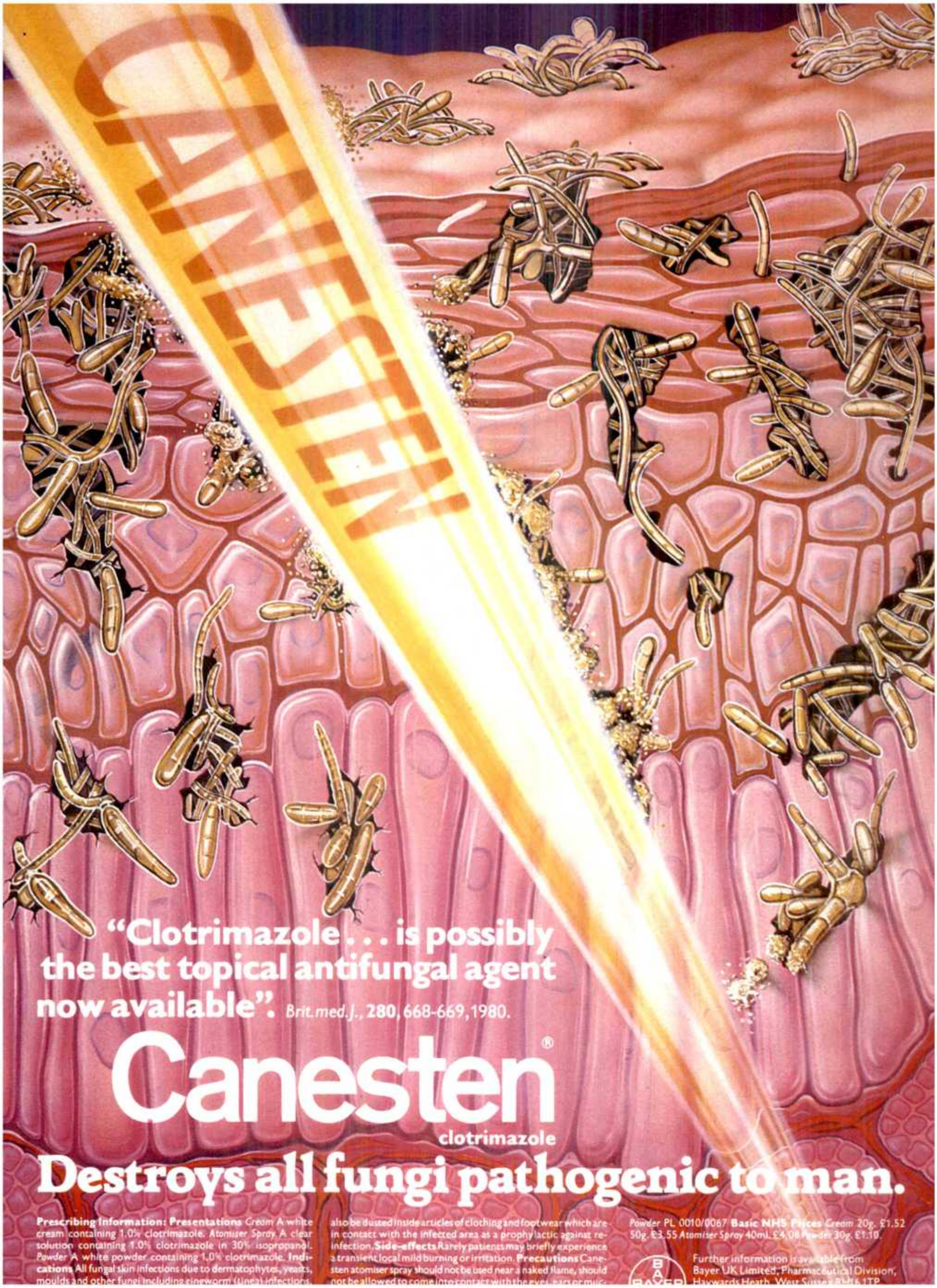
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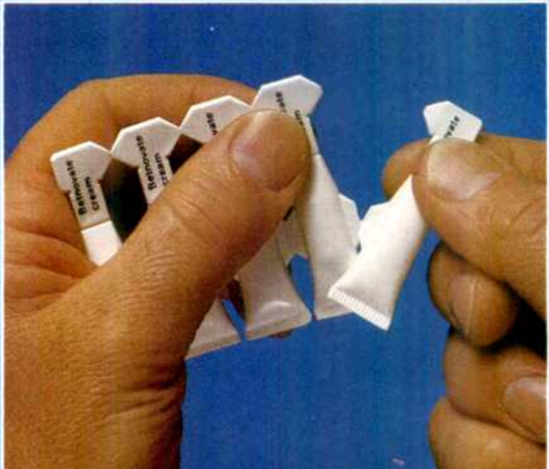

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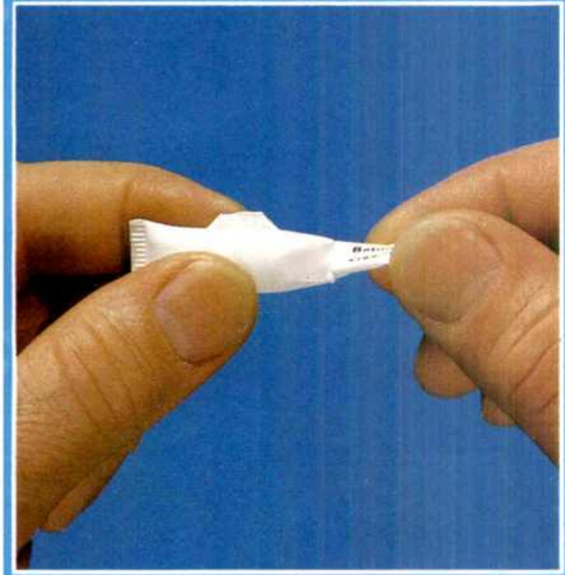

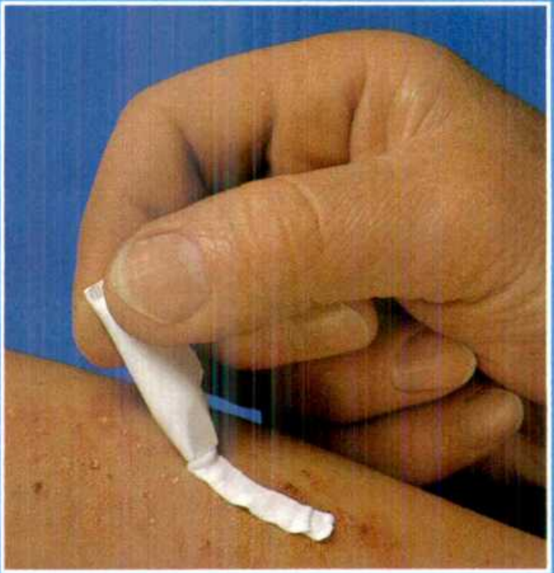

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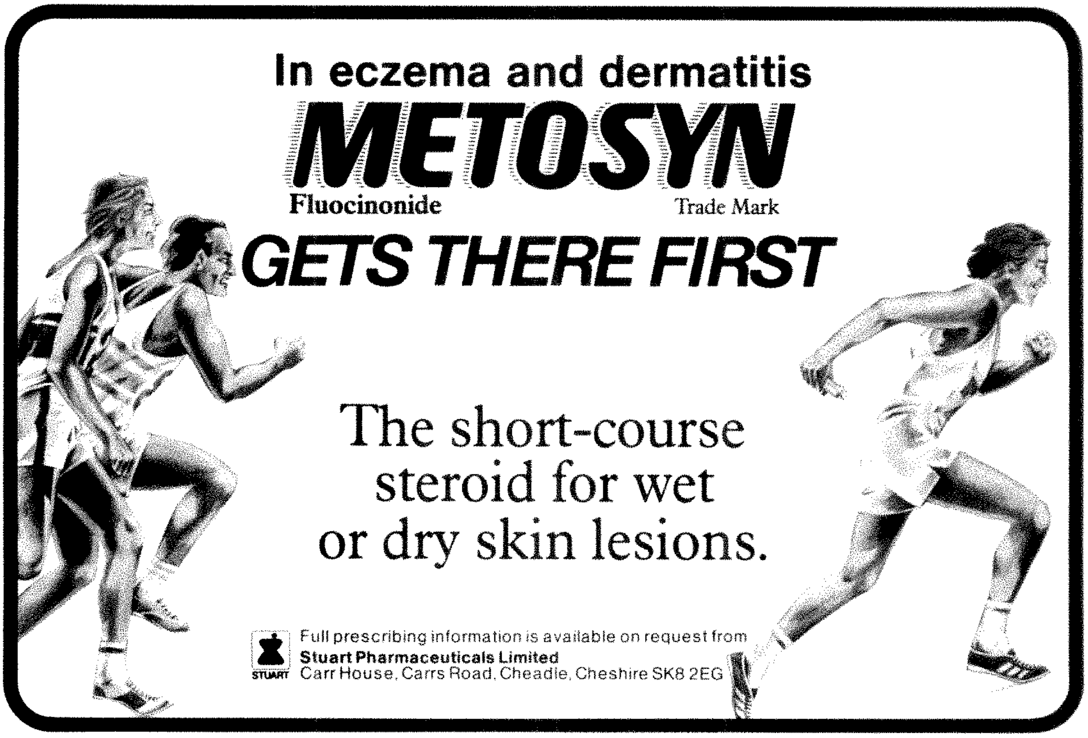

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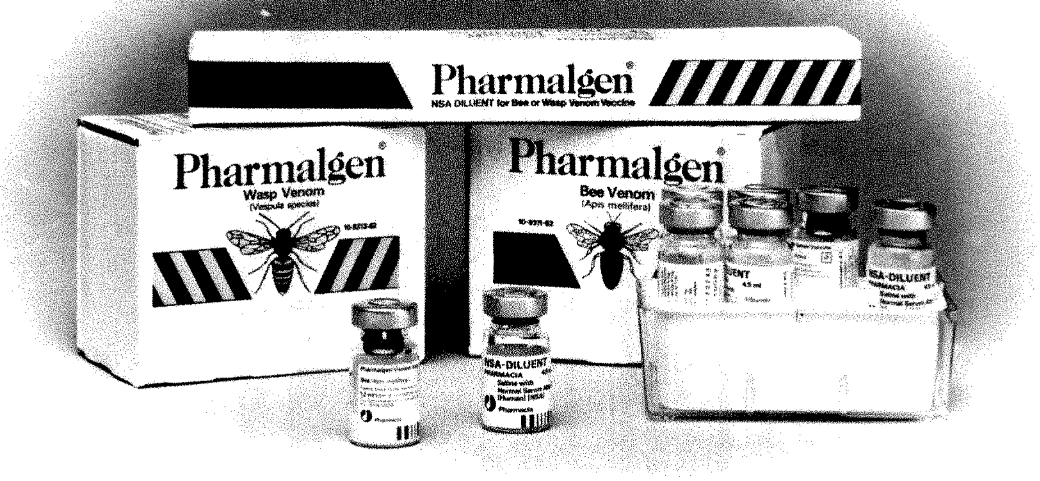

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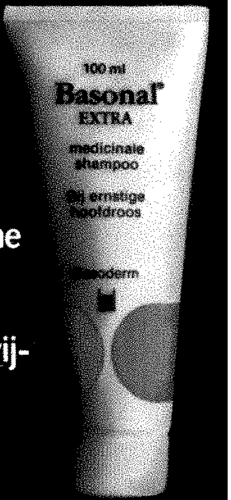

* 1. Veien, N. K. et al. Clin. Exp. Dermatol. (1979) 4, 53. 2. Pieterse, H. Multicentre trial, Technical Report (1980). 3. Lüpke, N. P. Ärztl. Kosmetol (1978) 8, 2

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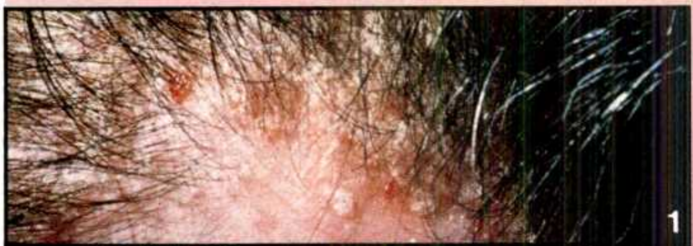

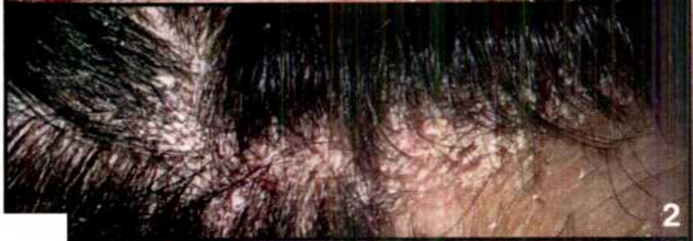

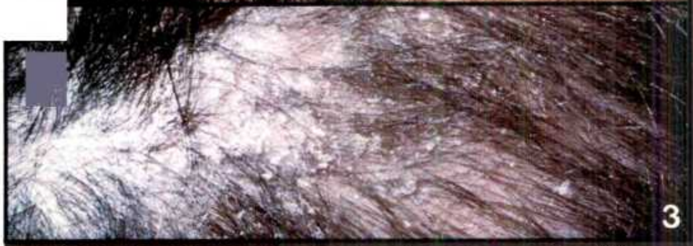

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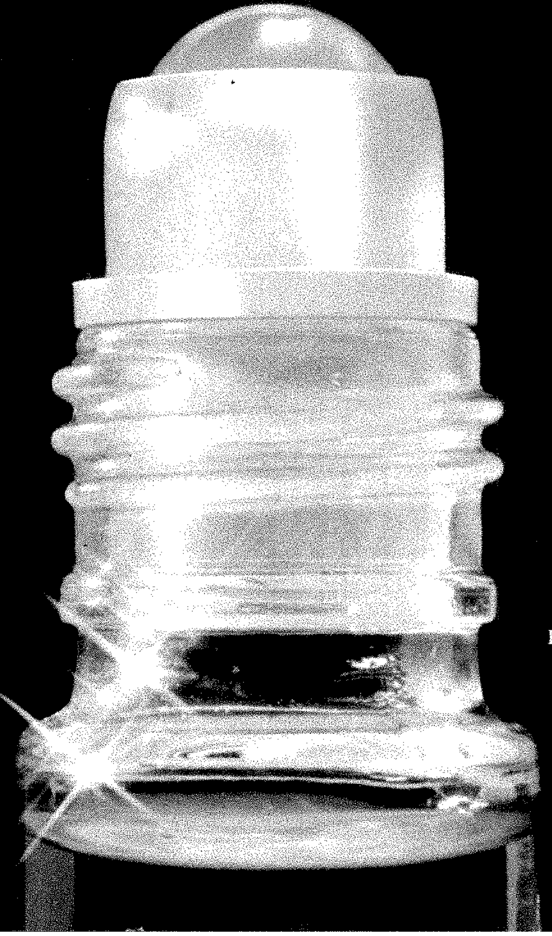

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Clinical and Laboratory Investigations

Evaluation of drug phototoxicity by photosensitization of *Trichophyton mentagrophytes*

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SUMMARY

Long wave ultraviolet radiation to suspensions of *T. mentagrophytes*, in the presence of photosensitizing chemicals, resulted in death of the fungus as measured by loss in colony-forming ability. Photosensitization could be demonstrated, with appropriate exposures and concentrations, with chlorpromazine, promethazine, perphenazine, anthracene, sulphanilamide, trimethylpsoralen, tolbutamide, tetrachlorosalicylanilide, and tribromosalicylanilide. No phototoxicity was detected with demethylchlortetracycline and thiazides.

The present system seems to be simple, reproducible, and sensitive as an in vitro screening test for the photosensitizing potential of chemicals.

Photosensitivity reactions are caused by a wide variety of chemical agents that are administered for therapeutic or diagnostic purposes. The photosensitizing potentialities of drugs have usually been discovered accidentally long after they have made their debut in clinical practice. Pre-clinical detection of adverse drug reactions is one of the most important subjects for medical investigation.

Several successful techniques have been presented for the detection of photosensitizing drugs in humans (Kligman & Breit, 1968; Kaidbey & Kligman, 1978a,b). However, it is not always possible to obtain human subjects for experimental study. Various *in vitro* and *in vivo* laboratory models have been used for evaluating phototoxic drug reactions in biological systems. Nevertheless, no one system has had complete success in predicting the clinical photosensitizing potential in man (Harber, Baer & Bickers, 1974a). Some of these systems may produce false positive responses while others may fail to detect commonly encountered phototoxic agents. Recently we reported the fungicidal effect of 8-methoxypsoralen (8-MOP) and subsequent long-wavelength ultraviolet radiation (UV-A) exposure on *Trichophyton mentagrophytes* (Horio & Watanabe, 1981). In the present study, we examined the photosensitizing effects of phototoxic compounds other than 8-MOP on suspensions of fungal spores. The purpose of this study was to investigate whether photosensitization of *T. mentagrophytes* can be

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used as a simple laboratory model for assaying phototoxic drug reactions *in vitro*. Various single-celled organisms including *Candida albicans*, viruses, tissue culture cells and bacteria have been used for this purpose (Daniels, 1965; Fowlks, 1959; Freeman, Murtishaw & Knox, 1970; Kobayashi, Wada & Mizuno, 1974).

MATERIALS AND METHODS

Preparation of the fungal suspensions

A zoophilic granular strain of *T. mentagrophytes* was grown on Sabouraud's dextrose agar slant medium for 2 weeks at 28°C. Suspensions were prepared by flooding the surface of the medium with sterile water and gently removing the colony with a wire loop. Clumps of removed fungus were ground with a mortar and pestle for 10 min and the suspensions were agitated vigorously for 10 min in a vortex mixer and filtered through 8 layers of gauze. The filtrates were washed with sterile water three times by centrifugation at 3,000 r.p.m. for 10 min. The spores in the preparation were counted using a haemocytometer and diluted to give desired densities.

Light source

The light source was a fluorescent black light (Toshiba FL 20S BLB, Toshiba Medical Supply, Tokyo) emitting rays between 300 and 420 nm (mainly UV-A peaking at 360 nm); five lamps were housed in a reflector unit. The irradiance of UV-A (315–400 nm) and UV-B (shorter than 315 nm) which was measured by a photovoltaic cell (UVR-305/365 Radio meter, Toshiba Medical Supply, Tokyo) through the plastic cover of a Petri dish, was 3·0 mW/cm^2 and 0·45 mW/cm^2, respectively, at a distance of 10 cm.

Photosensitizing chemicals

The drugs tested included chlorpromazine (CPZ), perphenazine, promethazine, demethylchlortetracycline (DMCT), anthracene, sulphanilamide, trimethylpsoralen, tolbutamide, tetrachlorosalicylanilide (TCSA), tribromosalicylanilide (TBS), hydrochlorothiazide, chlorothiazide, trichlomethiazide, and methyclothiazide. These materials were dissolved in either sterile water, ethanol, acetone, or benzene at concentrations of 1% and 0·1%. Trimethylpsoralen was insoluble in these solvents and was suspended in ethanol.

Photosensitization of fungal spores

Of each test solution, 0·25 ml was added to 1 ml of suspension containing 4×10^5 spores giving final concentrations of 0·2% and 0·02% test materials. Ten minutes after admixing, 0·01 ml of each mixture containing 3,200 spores was dropped onto Sabouraud's dextrose agar medium in Petri dishes (Terumo Schale $90^{\phi}\times 20$ cm, Terumo Co., Tokyo). Ten minutes after application, a 10·8 J/cm^2 exposure was given through the plastic cover of the Petri dish. After irradiation, plates were wrapped with aluminium foil and cultured at 28°C. Identical plates without photosensitizing compounds and irradiation, with only chemicals, with only irradiation, and with solvent and irradiation served as controls. The number of inoculated spores on control plates was the same as on test plates. These experiments were carried out in duplicate.

Influence of solvents

To compare the effect of solvents on photosensitization, CPZ was dissolved in sterile water, ethanol and acetone at a concentration of 0·01%. Photosensitization of fungal spores was evaluated with these solutions by the above mentioned procedure.

RESULTS

The results of culture for 1 week are summarized in Table 1. The plates which had not shown any fungal colony in 1 week did not reveal it thereafter. The control fungus suspensions in water revealed excellent growth. UV irradiation at a dose of 10·8 J/cm^2 showed a weak inhibitory effect on fungal growth even in the absence of photosensitizing compounds. On the other hand, UV irradiation showed a complete killing effect on fungus in the presence of promethazine, anthracene, (Fig. 1) sulphanilamide, and trimethylpsoralen. No detectable effect on fungal growth was observed with these chemicals in the absence of UV. Since 0·2% CPZ, 0·2% perphenazine, 0·2% and 0·02% TCSA, and 0·2% and 0·02% TBS showed a fungicidal effect even in the absence of UV-irradiation,

TABLE 1. Results of culture of *T. mentagrophytes* after photosensitization

Photosensitizer	Concentration	UV-A	no UV-A
None		+ +	+ + +*
Chlorpromazine	0·2%	–	–
	0·02%	–	+ + +
	0·002%	–	+ + +
Perphenazine	0·2%	–	–
	0·02%	–	+ + +
	0·002%	–	+ + +
Promethazine	0·2%	–	+ + +
	0·02%	–	+ + +
DMCT	0·2%	+ +	+ + +
	0·02%	+ +	+ + +
Anthracene	0·2%	–	+ + +
	0·02%	–	+ + +
Sulphanilamide	0·2%	–	+ + +
	0·02%	–	+ + +
Trimethylpsoralen	0·2%	–	+ + +
	0·02%	–	+ + +
Tolbutamide	0·2%	+	+ + +
	0·02%	+	+ + +
TCSA	0·2%	–	–
	0·02%	–	–
	0·002%	–	–, +
	0·0002%	–	+ + +
TBS	0·2%	–	–
	0·02%	–	–, +
	0·002%	–	+ + +
Hydrochlorothiazide	0·2%	+ +	+ + +
	0·02%	+ +	+ + +
Chlorothiazide	0·2%	+ +	+ + +
	0·02%	+ +	+ + +
Trichlomethiazide	0·2%	+ +	+ + +
	0·02%	+ +	+ + +
Methyclothiazide	0·2%	+ +	+ + +
	0·02%	+ +	+ + +

* Diameter of colony after one week culture.
+ + + >2 cm; 2 cm> + + >1 cm; 1 cm> + >0; – = no growth.

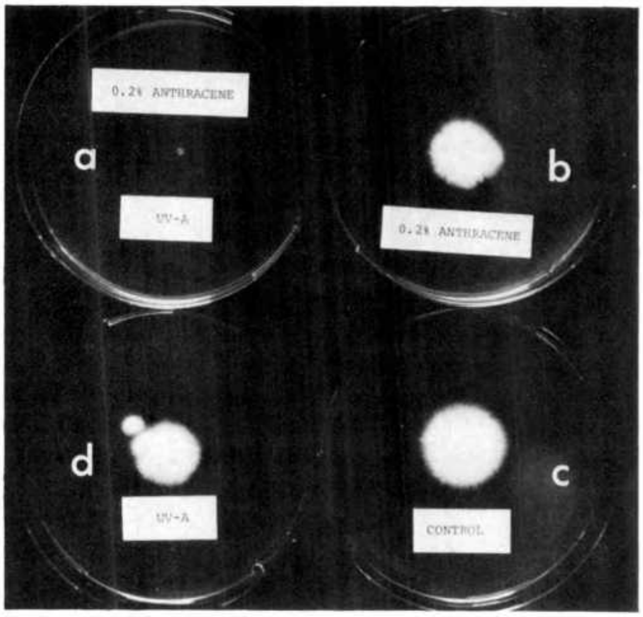

FIGURE 1. Results of 1-week culture of *T. mentagrophytes* after photosensitization: (a) 0·2% anthracene and UV-A; (b) anthracene alone; (c) no treatments (spore suspension in water); (d) UV-A exposure without anthracene.

lower concentrations of these compounds were also examined. At concentrations lower than 0·02% for CPZ and perphenazine, 0·0002% for TCSA, and 0·002% for TBS, UV-irradiation was necessary for the lethal effect of these compounds. In other words, photosensitization could be demonstrated also with these fungicidal chemicals. Tolbutamide and subsequent UV-irradiation as much as 10·8 J/cm^2 was insufficient to kill 3,200 spores completely, although an inhibitory effect on fungal growth was quite evident. DMCT, hydrochlorothiazide, chlorothiazide, trichlomethiazide, and methyclothiazide did not show inhibitory effects on fungal growths. These compounds apparently revealed a photosensitizing effect, but this was due to black light exposure alone. The light source emits primarily UV-A but also a small amount of UV-B.

The test substances which did not show a lethal photosensitizing effect were further examined using spore suspensions of lower densities. Tolbutamide killed 32 and 320 spores completely in the presence of UV-irradiation, while hydrochlorothiazide, chlorothiazide, trichlomethiazide, and methychlothiazide did not show any photosensitizing effect at all.

The intensity of CPZ photosensitivity did not vary with solvents. Any CPZ solution dissolved in either water, ethanol, or acetone revealed a similar photosensitizing effect.

DISCUSSION

UV-irradiation to suspensions of *T. mentagrophytes*, in the presence of various photosensitizing chemicals, resulted in death of the fungus as measured by loss in colony-forming ability. Neither UV alone nor chemical alone showed lethal effects on the fungus. Thus, phototoxicity was demonstrated in this system. At high concentrations, CPZ, perphenazine, TCSA, and TBS showed killing effects even in the absence of UV-irradiation. Therefore, such fungicidal compounds have to be examined for photosensitizing potential at lower concentrations. On the other hand, even in the presence of 0·2% tolbutamide, UV-irradiation as much as 10·8 J/cm^2 was insufficient to kill 3,200 spores completely, although an inhibitory effect on fungal growths was quite evident. To demonstrate complete lethal photosensitizing effects of such a weak photosensitizer, higher concentrations of chemical, higher exposure dose, or lower densities of fungal spores should be utilized. However, solubility of test substances is limited, and prolonged black-light exposure itself may have an inhibitory effect on fungal growth. In the present study, a lethal photosensitizing effect of tolbutamide could be demonstrated on 320 spores.

The present procedure using fungal suspensions appeared to be favourable to quantitative study of chemical photosensitivity. Daniels (1965) used *Candida albicans* on Sabouraud's medium to demonstrate the photosensitizing capacity of various compounds. This system has been shown to have many advantages. However, as Daniels mentioned, his paper-disk diffusion method seems to be inadequate to investigate photosensitizing power quantitatively. In contrast with Daniel's results in *Candida albicans*, phototoxicity of sulphanilamide was easily demonstrated using *T. mentagrophytes*. Kahn & Fleischaker (1971) evaluated the phototoxic potential using blood cell haemolysis by photosensitizing compounds. In their system, there was considerable variation in photoreaction depending on the solvent. In the present procedure, the photosensitizing effect of CPZ did not vary with the solvents examined.

A variety of procedures has been used for assaying phototoxic drug reactions in biological systems. A comprehensive review of laboratory models was presented by Harber *et al.* (1974a). Some of these may produce false positive responses while others may fail to detect well known clinical photosensitizers. Although the present study demonstrated the phototoxic potential of many drugs, DMCT and thiazides revealed no detectable photosensitizing effect in this system. This discrepancy with clinical experience remains unexplained. The *in vitro* models eliminate metabolism of the drug in the liver or in other organs. Therefore, the system using single-cell organisms cannot detect phototoxicity of the chemicals whose metabolite is the important photosensitizer. Furthermore, these systems may be unsuitable for assaying photosensitizing chemicals when the action spectrum is less than 315 nm, because of a lethal effect of the radiation itself. In the present study, black-light exposure had some inhibitory effect on fungal growth even in the absence of photosensitizers. However, this may not have been caused by UV-A, but by a small amount of UV-B which is emitted from the light source. The cover of the plastic Petri dish utilized in the present study cuts off only 30% of UV-B. Fortunately, photosensitizing reactions due to exogenous agents are usually caused by UV-A or sometimes by visible light (Morikawa *et al.*, 1976). Therefore, UV-B would have been better eliminated completely by window glass or other plastic films. Phototoxicity due to rose bengal could be demonstrated also by the present procedure using visible light (unpublished observations).

The mechanism of the lethal effect of photosensitizing chemicals on fungus is not clear. The primary subcellular level for phototoxic damage may involve the nucleus, cytoplasmic organelles, and the cell membrane (Harber & Baer, 1972; Harber, Baer & Bickers, 1974b). The present system detected nuclear phototoxicity (8-MOP and trimethylpsoralen) (Musajo, & Rodighiero, 1967), cytoplasmic phototoxicity (anthracene) (Allison, Magnus & Young, 1966), and also cell membrane phototoxicity (rose bengal) (Allison *et al.*, 1966).

Since no single system so far developed has had complete success in predicting the clinical photosensitizing potential of chemicals, a combination of various systems is necessary for assaying new drugs which are not known for their photosensitizing ability. The present procedure appears to be adequate as one of these systems, since it is simple, reproducible and sensitive. Neither specialized technique nor equipment is necessary.

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ACKNOWLEDGMENT

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British Journal of Dermatology (1981) **105**, 371–382.

A previously undescribed condition: tricho-odonto-onycho-dermal syndrome. A review of the tricho-odonto-onychial subgroup of ectodermal dysplasias

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SUMMARY

An apparently hitherto undescribed ectodermal dysplasia/malformation syndrome is presented. The patient, the last son in an outbred sibship of four males, presents scalp hypotrichosis, aplasia cutis congenita of the scalp, dental abnormalities, onychodysplasia, dry skin with hypochromic and atrophic (poikiloderma-like) spots with vicarious (marginal) hyperchromia, unusual facies, asymmetrical skull, absent right nipple, irregular areolae, palmar keratosis, dermatoglyphic alterations, syndactyly, clinodactyly, phalangeal aplasias and hypoplasias, right leukoma, abnormal EEG, and other findings. The aetiology is unknown. A review of seventeen ectodermal dysplasias is presented for differential diagnosis.

Ectodermal dysplasias, which in the broad sense include both pure ectodermal dysplasias and ectodermal dysplasia/malformation syndromes, form a large nosological group (Freire-Maia, 1971, 1977; Witkop, Brearley & Gentry, 1975; Pinsky, 1975, 1977). For differential diagnosis, didactic, research, and bibliographical retrieval purposes, they have been classified into different subgroups according to the presence or absence of (1) trichodysplasia, (2) dental abnormalities, (3) onychodysplasia, and (4) dyshidrosis (Freire-Maia, 1971, 1977). Dyshidrosis (defective sweating) includes anhidrosis, hypohidrosis and hyperhidrosis. Group A includes conditions with at least two of these signs and group B those with at least one of them plus at least another sign of ectodermal origin. Conditions from both groups may present, however, other signs with the same or another embryological origin as well as malformations. Group A encompasses subgroups commonly referred to either with the aid of combinations of numbers 1–4 or of terms of Greek origin related to the above mentioned signs. Therefore, group A should present eleven theoretically expected subgroups: 1-2-3-4, 1-2-3, 1-2-4, 1-3-4, 2-3-4, 1-2, 1-3,

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1-4, 2-3, 2-4 and 3-4, respectively, also termed tricho-odonto-onycho-dyshidrotic, tricho-odonto-onychial, tricho-odonto-dyshidrotic, tricho-onycho-dyshidrotic, odonto-onycho-dyshidrotic, tricho-odontial, etc. Freire-Maia (1971) classified thirty-two conditions into eight subgroups, and Freire-Maia (1977), about fifty-seven into ten subgroups. Our most recent review (Freire-Maia & Pinheiro, 1981) includes about eighty conditions in the same ten subgroups (no condition classified as 3-4 has so far been described as far as we know).

This paper presents a previously undescribed ectodermal dysplasia/malformation syndrome of the 1-2-3 subgroup, for which we suggest the name tricho-odonto-onycho-dermal (TOOD) syndrome. A review of seventeen ectodermal dysplasias belonging to this subgroup is presented for the purpose of differential diagnosis.

CASE REPORT

The propositus, a boy of Italian and mixed Brazilian origin, was born on 27 January 1968 after an uncomplicated 8 months pregnancy and normal delivery, to a gravida 8, para 6, abortus 2 mother (who underwent thyroid surgery after 2 months of pregnancy). Birth weight was 1·900 kg. Besides the anomalies that will be mentioned below, he had hypertrophy of the frenum linguae that was surgically repaired.

The sibship has four males, the propositus being the only one affected in the whole family. His non-consanguineous mother and father were 37 and 39 years old, respectively, at the time of his birth.

Physical examination at 12 years of age revealed a height of 137 cm (below the 10th percentile), a weight of 31 kg (below the 25th percentile), and an occipitofrontal head circumference of 52 cm (below the 25th percentile of the Tanner and Whitehouse chart). A dry skin with hypochromic, atrophic, and poikiloderma-like spots (some with telangiectases) was noted over the whole body (Fig. 1). In cold weather, bleeding fissures (mainly over joints) and subsequent infection tended to appear. Skin biopsies from the thorax and nose revealed focal areas of hyperkeratosis and an increased amount of pigment in the epidermis. The right nipple was absent, and both areolae presented irregular outlines (Fig. 1). Chest circumference (CC) at

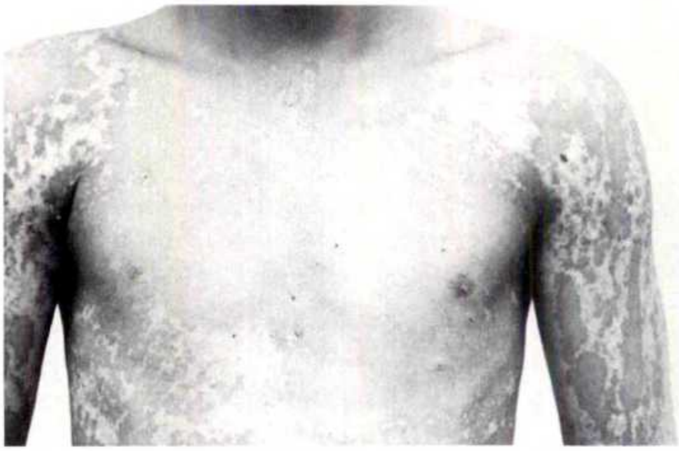

FIGURE 1. Hypochromic, atrophic and poikiloderma-like spots, absence of right nipple, and both areolae with irregular outlines.

the nipple level—71 cm; internipple distance (ID)—16·5 cm; ID/CC—0·23; all of them were roughly around the 50th percentile (Feingold & Bossert, 1974). The palms showed mild hyperkeratosis.

The craniofacial features consisted of hypotrichosis (without structural abnormalities at the level of light microscopy) in the parieto-occipital region; an area of alopecia in the centro-parietal region due to aplasia cutis congenita; mild asymmetry of the skull; scanty and downward pointing eyelashes of the upper lids; sparse and irregular eyebrows especially in their distal 2/3; long philtrum; hypoplastic right ala nasi; microstomia with thin lips; vicarious (marginal) hyperpigmentation; periorbital and palpebral hyperchromia; mild micrognathia; right palpebral ptosis; and cup ears (Fig. 2).

Dermatoglyphic analysis showed an excess of white lines and a transverse palmar crease in each palm, as well as a mosaic patchy distribution of dermal ridges both in the palms and in the fingertips, i.e. there were regions with normal ridges side by side with regions completely devoid of them (Fig. 3). Due to this disturbance, it was impossible to identify the dermatoglyphic finger patterns, to calculate TRC, to determine atd angle, etc.

Ophthalmological examination revealed leukoma in the right eye. Fundoscopy was normal. The inner intercanthal distance was 3 cm, the interpupillary distance 5 cm, and the outer intercanthal distance 7.5 cm; these are within the normal ranges (Feingold & Bossert, 1974).

Oral findings (Fig. 4) showed $\frac{1|}{4|}$ to have enamel hypoplasia, $\frac{7|}{321|123}$ missing ('congenitally' absent), $\frac{8|}{8|8}$ tooth buds missing, $\overline{|3\,5}$ to be persistent deciduous, $\underline{6|}$ to have alterations of size and form, and $\frac{5|3}{|5}$ to have delayed eruption. Also, two extranumerary teeth were noted: one left upper canine and one central upper incisor.

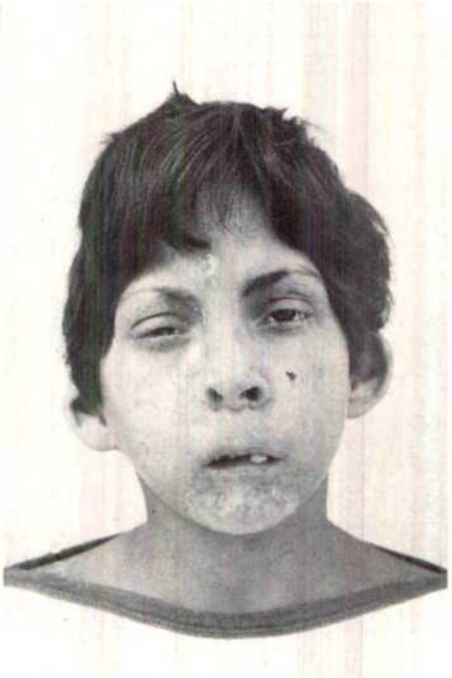

FIGURE 2. Cup ears, microstomia with thin lips, hypoplastic right ala nasi, right palpebral ptosis, long philtrum, scanty eyelashes, sparse eyebrows especially at their distal two-thirds, hypochromic spots, marginal hyperpigmentation, and periorbital and palpebral hyperchromia.

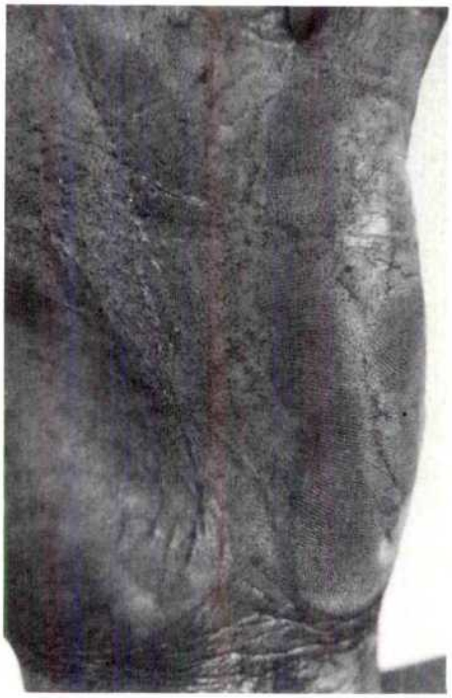

FIGURE 3. Left palm (stained with a 5% solution of o-phthaldialdehyde in xylene) presenting mosaic patchy distribution of dermal ridges, and a transverse palmar crease.

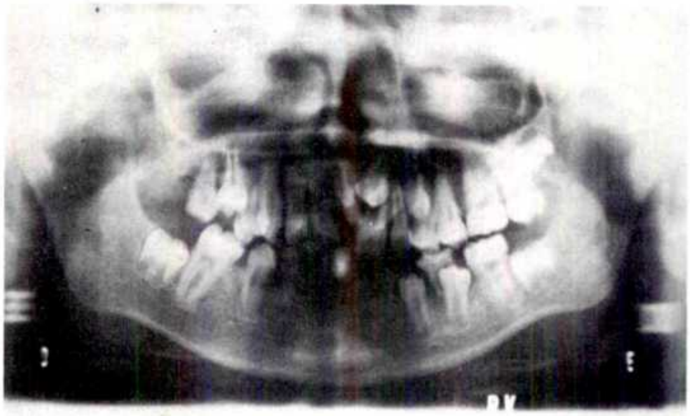

FIGURE 4. Orthopantomogram showing hypodontia, persistent deciduous, delayed eruption, and extranumerary teeth.

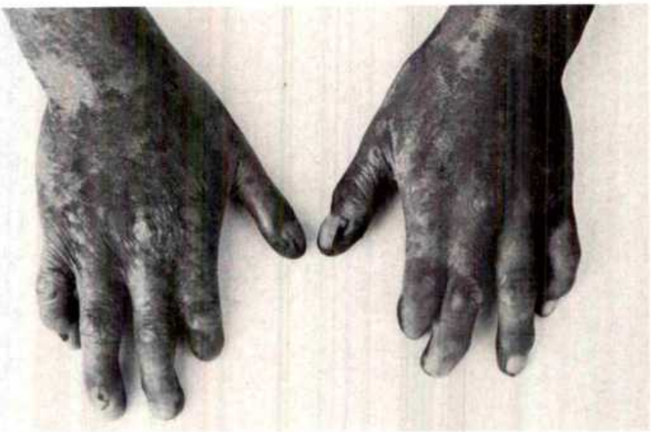

FIGURE 5. Hypochromic spots, wrinkled skin, syndactyly, clinodactyly, absence of three nails, presence of two normal and five dystrophic nails, short index fingers, and hypoplastic and flexionless right thumb.

Of the finger- and toe-nails, fifteen were severely dystrophic and three absent (the 4th and 5th left fingernails were normal). The following abnormalities were also noted: bilateral clinodactyly of both fifth fingers; syndactyly of the 2nd and 3rd fingers of both hands, of the 2nd and 3rd toes of both feet, and of the 4th and 5th left toes; hypoplastic and flexionless right thumb; wrinkled backs of hands (Figs 5 and 6), and pronounced *manus cava*.

X-rays of hands and feet revealed hypoplastic distal and middle phalanges of both 2nd fingers, and absent middle phalanges of all the toes. The rest of the skeleton was normal.

EEG suggested a bilateral fronto-temporal irritating process.

Intelligence, hearing, blood count, urinalysis, sweating capacity, intravenous pyelogram, genital system, and karyotype were normal.

COMMENTS

Differential diagnosis must be made between the present case and the seventeen other conditions known in the tricho-odonto-onychial subgroup (Table 1). The TOOD syndrome is

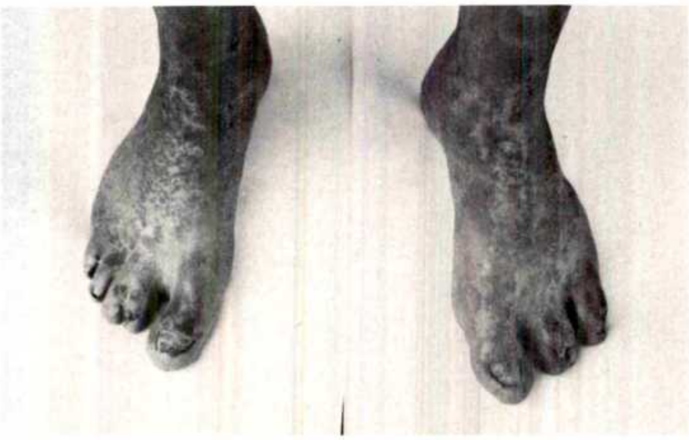

FIGURE 6. Hypochromic spots, one right and two left syndactylies, and dystrophic nails.

TABLE 1. Tricho-odonto-onychial subgroup of ectodermal dysplasias

Condition and reference	Hair	Teeth	Nails	Skin	Genetics and other findings
1. Rothmund-Thomson syndrome or poikiloderma congenitale (Kraus, Gottlieb & Meliton, 1970; Kristensen, 1975)	Hypotrichosis; occasionally alopecia	Hypodontia; microdontia; supernumerary teeth; pronounced caries; delayed eruption	Frequently dystrophic	Poikilodermatous lesions with atrophy, irregular pigmentation and telangiectases; palmoplantar hyperkeratosis; sensitivity to sunlight	AR; cataract; short stature; skeletal anomalies of the limbs; hypogonadism; cryptorchidism; skull abnormalities
2. Hidrotic ectodermal dysplasia or Fischer-Jacobsen-Clouston syndrome (Fischer, 1910; Jacobsen, 1928; Clouston, 1929)	Dry, fine and blonde; slow growth; hypotrichosis	Hypodontia; anodontia; widely spaced; natal teeth; carious teeth	Thickened and slightly discoloured; striated; paronychia; convex ends; anonychia; tendency to horny hypertrophy	Dry and rough; tendency for scaliness; hyperpigmentation of some areas, thick dyskeratotic palms and soles	AD; strabismus; cataract; myopia; opaque patches in the retina; tufting of terminal phalanges; clubbing of fingers; speech difficulties; thickening of the skull bones
3. Coffin-Siris syndrome (Coffin & Siris, 1970; Carey & Hall, 1978)	Sparse scalp hair; bushy eyebrows and lashes; hirsutism of limbs, forehead and back	Delayed eruption; microdontia	Absent to hypoplastic fifth fingernails and toenails; other nails occasionally hypoplastic or absent	Dermatoglyphic changes	AD (?); thick lips; wide mouth and nose; anteverted nostrils; low nasal bridge; microcephaly; psychomotor and growth retardation; lax joints; clinodactyly; small or absent patellae; frequent respiratory infections; absence of terminal phalanges of fifth fingers and toes; aplasia or hypoplasia of middle and proximal phalanges of other fingers and toes

	Condition and reference	Hair	Teeth	Nails	Skin	Genetics and other findings
4.	Odontotrichomelic syndrome or tetramelic ectodermal dysplasia (Freire-Maia, 1970; Freire-Maia *et al.*, 1970; Cat, Costa & Freire-Maia, 1972; Rapone-Gaidzinski, 1977)	Severe hypotrichosis	Hypodontia; microdontia; coniform teeth; persistence of deciduous teeth	Hypoplastic (?)	Thin, dry, and shiny; dermatoglyphic disturbances; hypoplastic nipples; hypoplastic or absent areolae; wrinkles in the face	AR (?); protruding lips; enlarged nose; deformed auricles; cleft lip; extensive tetramelic aplasias; EEG and ECG abnormalities; growth retardation; metabolic abnormalities
5.	Tricho-dento-osseous syndrome or enamel hypoplasia with curly hair (Robinson, Miller & Worth, 1966; Jorgenson & Warson, 1973)	Dry, thick, tough, and with short curls	Small, pitted, and widely spaced; hypoplastic enamel; taurodontism; proneness to caries	Flat, thickened, misshapen, and striated; tendency to peeling and breaking off	Normal	AD; sclerosteosis most evident in the skull
6.	Incontinentia pigmenti or Bloch-Sulzberger syndrome (Curth & Warburton, 1965; Carney, 1976)	Thin; alopecia in about one-third of cases	Hypodontia; anodontia; peg-shaped; delayed eruption	Dystrophic in about one-tenth of cases	Neonatal vesiculo-bullous eruption followed or accompanied by verrucous lesions and bizarre pigmentation	XD with intrauterine lethality of males (?); ophthalmological and CNS alterations in about one-third of cases; skeletal changes (hemivertebrae, cervical ribs, etc.) in about one-fifth of cases
7.	Cranioectodermal dysplasia or Sensenbrenner-Dorst-Owens syndrome (Sensenbrenner, Dorst & Owens, 1975; Levin *et al.*, 1977)	Thin, sparse and slow growing; abnormal structure	Microdontia; hypodontia; widely spaced; enamel hypoplasia; fusion (deciduous teeth)	Broad and short	Dimples over elbows and knees; dermatoglyphic disturbances	AR (?); hyperopia; myopia; nystagmus; frontal bossing; epicanthal folds and antimongoloid slant; full cheeks; everted lower lip; hypotelorism; abnormal auricles; broad nasal bridge; anteverted nares; hypotonia; rhizomelia; syndactyly; clinodactyly; dolichocephaly; osteoporosis; CHD

	Condition and reference	Hair	Teeth	Nails	Skin	Genetics and other findings
8.	Fried's tooth and nail syndrome (Fried, 1977)	Fine and short; scanty eyebrows	Hypodontia; peg-shaped	Thin fingernails; small, thin, and concave toenails	Normal	AR; prominent lips and chin; cleft lip; branchial cyst on the neck
9.	Hypodontia and nail dysgenesis or tooth and nail syndrome (Witkop, 1965; Giansanti, Long & Rankin, 1974; Witkop *et al.*, 1975)	Fine and brittle	Hypodontia; widely spaced; cone-shaped; occasionally anodontia and supernumerary teeth	Small and spoon-shaped; slow growth in children; longitudinal ridging	Occasionally dry; premature wrinkles on the face	AD; everted lips
10	Dento-oculo-cutaneous syndrome (Ackerman, Ackerman & Ackerman, 1973a,b)	Scanty with vellus hairs in the moustache and beard areas	Taurodont, pyramidal, or fused molar roots	Horizontal ridging of the fingernails with distal onychoschizia	Indurated and hyperpigmented over the interphalangeal joints of the fingers	ASD (?); juvenile glaucoma; upper lip with absence of the 'Cupid's bow'; thickening and widening of the philtrum; ectropion of both lower eyelids; syndactyly; clinodactyly
11.	Tricho-rhino-phalangeal syndrome —Type I (Giedion, 1966; Giedion *et al.*, 1973)	Fine, usually blonde and sparse; slow growing; medially thick and laterally sparse or absent eyebrows	Supernumerary incisors; microdontia; poorly aligned	Thin, short and with striae; flattened; koilonychia; racket thumb nails	Not mentioned (probably normal)	Heterogeneity: AD and AR (?); pear-shaped nose; long and wide philtrum; small stature; brachymesophalangy; brachymetacarpy; brachymetatarsy; type 12 cone-shaped epiphyses
12.	Ellis-van Creveld syndrome or chondro-ectodermal dysplasia (Ellis & van Creveld, 1940; Pilotto, 1979)	Thin, brittle, sparse, and hypochromic	Neonatal; precocious exfoliation; hypodontia; occasionally hypoplastic enamel	Dysplastic	Occasionally different alterations (hypotrophy, flaccidity, eczema, etc.)	AR; broad nose; short-limb dwarfism; bilateral post-axial polydactyly; brachymetacarpy; fusion of the hamate and capitate; CHD; gingivolabial fusion; respiratory difficulties

Condition and reference	Hair	Teeth	Nails	Skin	Genetics and other findings
13. Cystic eyelids, palmoplantar keratosis, hypodontia and hypotrichosis or Schöpf-Schulz-Passarge syndrome (Schöpf, Schulz & Passarge, 1971)	Hypotrichosis	Extensive hypodontia	Frail with longitudinal and oblique furrows	Palmoplantar keratosis	AR; bilateral early cataract; arteriosclerotic fundi; myopia; cysts of eyelids developing late
14. Šalamon syndrome (Šalamon & Miličevič, 1964; Šalamon, Čubela & Bogdanović, 1967; Šalamon, Lazović & Stenek, 1972)	Dry, inelastic, wire-like, lustreless, and sparse with telogen effluvium in the scalp; pili torti; trichorrhexis nodosa	Hypodontia; microdontia	Highly dystrophic; brittle; ungues plicatae; toenails more severely affected	Tendency to develop warts and papules; hyperkeratotic scalp	AR; chronic blepharoconjunctivitis; punctate keratitis; atrophia strati pigmenti retinae; madarosis et trichiasis palpebrae; maculae cornea centrales et periphericae; piriform nose; slight osteoporosis of the arms and legs
15. Tricho-oculo-dermo-vertebral syndrome (Alves *et al.*, 1980, 1981)	Dry and rough; hypotrichosis	Tendency to develop caries; spaced incisors; gingivitis	Thin and brittle fingernails; wide, short and dystrophic toenails with paronychia	Dry with tendency to fissures and infection; scaling hyperchromic (ichthyosiform-like) spots; hyperkeratosis; thick corneal layer	AR; bilateral nuclear cataract; narrow palpebral fissures; entropion; trichiasis; hypoplasia of nasal alae; micrognathia; wide nasal bridge; enlarged interphalangeal joints; minor skin webbing of fingers; dermatoglyphic changes; genua valga; cubitus valgus; manua cava; kyphoscoliosis; spina bifida occulta

Condition and reference	Hair	Teeth	Nails	Skin	Genetics and other findings
16. Oculo-dento-digital syndrome II (O'Rourk & Bravos, 1969)	Hypotrichosis	Enamel hypoplasia; microdontia; hypodontia; malocclusion; conical incisors	Dysplastic	Normal	?; microphthalmia; esotropia; nystagmus; atrophy of the iris; anteversion of the nostrils; small alae nasi; microstomia with furrowing and overlapping of the upper lip; micrognathia; polydactyly; camptodactyly; syndactyly; hypophalangy
17. Arthrogryphosis and ectodermal dysplasia (Côté, Adamopoulos & Pantelakis, 1981)	Hypotrichosis; oval hair cross-section revolving on its own axis ('stretched pili torti')	Enamel hypoplasia	Absent at birth; tendency to longitudinal breaks	Dry; tendency to excessive bruising and scarring after injuries and scratching	?; epicanthus; mongoloid slant; small stature; clinodactyly; syndactyly; talipes equinovarus; arthrogryposis of all joints
18. Tricho-odonto-onycho-dermal (TOOD) syndrome (this paper)	Hypotrichosis	Hypodontia; persistence of deciduous; enamel hypoplasia; delayed eruption; supernumerary teeth; abnormal shape and size	Severely dystrophic; anonychia in some fingers	Dry with hypochromic, atrophic, and poikiloderma-like spots; absent nipple; irregular areolae; palmar keratosis; dermatoglyphic changes; aplasia cutis congenita of the scalp	?; leukoma; long philtrum; cup ears; microstomia with thin lips; hypoplastic ala nasi; palpebral ptosis; micrognathia; hyperpigmented eyelids and periorbital regions; clinodactyly; syndactyly; hypoplastic and flexionless right thumb; manus cava; hypoplastic distal and middle phalanges; congenital hypertrophy of the frenum linguae

AR—autosomal recessive; AD—autosomal dominant; ASD—autosomal semi-dominant; XD—X-linked dominant; ?—unknown or doubtful.

clearly different from all of them and is at the first stage of definition of a new syndrome—the physical examination stage (Opitz, Herrmann & Dieker, 1969).

The TOOD syndrome may have a genetic aetiology, but our data do not provide a clear suggestion of any pattern of inheritance.

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A histometric and autoradiographic study of hydrocortisone action in cultured human epidermis

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SUMMARY

Pieces of human skin maintained their viability, grew and differentiated for at least 10 days in chemically defined (serum-free) culture conditions. Physiological concentrations of hydrocortisone, added to the cultures, were shown to cause thinning of epidermis, which was due to decreased rate of epidermal proliferation. The relative volume of normal, orthokeratotic stratum corneum with underlying granular cells was increased concomitantly with epidermal thinning. The present findings show that even small concentrations of the hormone influence epidermal cell proliferation and suggest that hydrocortisone has a physiological role in keratinization. The possibility of using human skin culture in the assessment of the antiproliferative and atrophogenic action of synthetic glucocorticoids is discussed.

Synthetic corticosteroids have been widely used in dermatology because of their anti-inflammatory potency. They also exert, when administered in pharmacological doses, antimitotic actions on human epidermis (Fisher & Maibach, 1971; Fry & McMinn, 1968). When the antimitotic and anti-inflammatory capacities of these steroids are linked, thinning of the epidermis, which is explained in terms of cell proliferation, is an unavoidable side-effect of therapy (Winter & Burton, 1974). Potent corticosteroids are also known to normalize keratinization in psoriatic plaques (Fry & McMinn, 1968).

Physiological glucocorticoids such as hydrocortisone have also been shown to be antimitotic in epidermis (Reaven & Cox, 1968; Marks, Pongsehirum & Saylan, 1973) although contradictory findings have been made (Fisher & Maibach, 1971; Laurence & Christophers, 1976). The antimitotic action of hydrocortisone seems to be dose-dependent, and rather high concentrations are needed (Reaven & Cox, 1968; Marks *et al.*, 1973). Furthermore, evidence derived from animal experiments suggests a specific role for hydrocortisone in epidermal cell differentiation

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(Sugimoto & Endo, 1969). Epidermal transglutaminase (Obinata & Endo, 1977) and DNA-dependent RNA polymerase activity (Lukacs & Braun-Falco, 1972) are induced by hydrocortisone in epidermis, and it has been hypothesized that these enzymes participate in keratinization. It has even been postulated that the hydrocortisone-induced atrophy in mice is rather a consequence of accelerated keratinization than of decreased proliferation (Laurence & Christophers, 1976).

The possible role of hydrocortisone in normal growth and differentiation of human epidermis is obscure. The present study was undertaken to evaluate the effects of hydrocortisone at physiological concentration in human skin culture, in which epidermal proliferation and differentiation are known to take place (Tammi & Jansén, 1980).

MATERIALS AND METHODS

Skin culture

Skin samples were obtained from nine female patients, aged 38–75 years, undergoing mastectomy operation. Skin strips were taken immediately after operation into culture medium. The subcutaneous fat and most of the dermal connective tissue were trimmed away. Pieces (explants) of about 2·5 mm^2 and 0·5 mm thick were prepared.

The organ culture method of Trowell (1959) was followed with slight modifications described earlier by us (Tammi & Jansén, 1980). The culture medium was Eagle's minimal essential medium (modified) with Earle's balanced salt solution (Flow Laboratories Ltd) buffered with 20 mM Hepes and 25 mM bicarbonate and supplemented with 50 iu/ml of sodium penicillin G and 50 μg/ml of streptomycin sulphate. No serum was added to the medium in order to avoid even trace amounts of other hormones. The medium was renewed every second day. The culture dishes were kept in airtight chambers containing a gas mixture of 40% oxygen, 5% carbon dioxide and 55% nitrogen. The chambers were regassed for 10 min daily.

Hydrocortisone (Hydrocortisone sodium succinate, Orion, Mankkaa, Finland), dissolved in a small volume of medium, was added to give the final concentrations of 10^{-9} mol/l, 10^{-7} mol/l and 10^{-5} mol/l at the start of the culture and after each renewal of medium.

Histological examination

Explants for histological processing were harvested before culture and at different times after the beginning of culture. They were fixed in neutral buffered formaldehyde, dehydrated in alcohol and embedded in paraffin. Thirty serial sections (7 μm) perpendicular to the epidermal surface were cut and stained with haematoxylin and eosin.

The parameters in Fig. 1 were measured by a histometric recording method (Tammi, Jansén & Santti, 1979). In every experiment (one experiment per patient) six to eight explants from hormone-treated and control cultures were taken for analysis on each day. Data from eight experiments were pooled for the calculation of means and standard errors of the means.

DNA synthesizing cells in epidermis were labelled with ^{3}H-thymidine [(6-^{3}H)-thymidine, TRK 296, specific activity 28 Ci/mmol, The Radiochemical Centre, Amersham, U.K.] in three experiments, each including control and hormone-treated cultures. Six explants were harvested on days 1, 2, 3, 4, 5, and 6 and incubated for 2 h at 37°C in a culture medium with 5 μCi/ml of tritiated thymidine. After incubation the explants were washed twice with cold medium, fixed, dehydrated and embedded in paraffin. Sections 5 μm thick were cut perpendicular to the epidermal surface and dipped with nuclear emulsion NTB 3 (Eastman Kodak Co., Rochester, N.Y.). The autoradiograms were exposed for 14 days at −15°C, developed in Kodak D-19

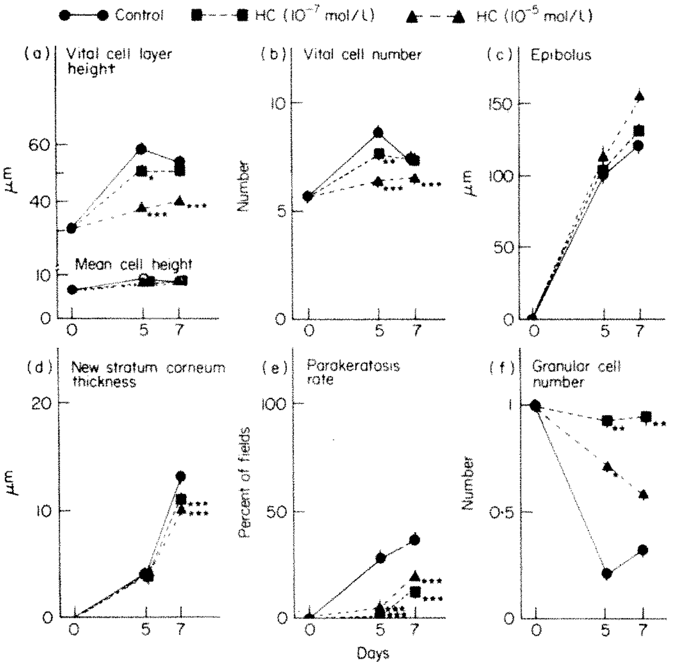

FIGURE 1. The effect of hydrocortisone on the histometric parameters of human skin in organ culture: means and standard errors of the means are calculated from the combined data of eight experiments. Significant differences between the means of control cultures and hormone-treated cultures are indicated as follows: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$. HC = Hydrocortisone, MCH = mean cell height in epidermis (vital cell layer thickness/number of vital cell layers).

developer (Kodak Ltd, London, U.K.) for 4 min and stained with haematoxylin and eosin. The number of labelled nuclei per 1 mm of the epidermis was counted in six sections (at least 30 μm apart) of every explant. Data from three experiments were pooled for calculations of the means and standard error of the means.

The transit time of epidermal cells from the basal layer to the granular layer was measured by incubating explants on the first culture day with 5 μCi of tritiated thymidine/ml medium for 4 h. After incubation, the explants were washed with fresh medium and transferred to the original culture dishes. On days 4, 6 and 8 after the thymidine labelling seven to nine explants were harvested from control and hydrocortisone-treated cultures, fixed and processed for autoradiography as described above. The position of the labelled cells was analysed by counting the number of the vital cell layers below and above the labelled cells. In each explant the position of at least fifteen labelled cells was analysed. Means and standard errors of the means were calculated from pooled data of three experiments.

Statistical methods

The histometric and autoradiographic data were subjected to a three-way analysis of variance (HYLPS-program, Univac) of the random effect of patients and fixed effects of culture periods, and hormone-treatments. For the transit time experiments a two-way analysis of variance was performed. Comparisons between means at different hormone concentrations were carried out using a simultaneous confidence limits test procedure by Newman-Keuls (Winer, 1971).

RESULTS

Human epidermis showed normal growth and architecture over the whole culture period used in the present study. The height of vital epidermis as indicated both by the thickness and by the number of cell layers increased during the culture (Fig. 1a and b). The vitality of the epidermis remained good until the seventh day of culture. Approximately 1% of the epidermal area was necrotic on the seventh culture day. Keratinization of upper cell layers continued in culture, too, but was subject to changes (Fig. 1d and e). The number of granular cells was considerably decreased (Fig. 1f), and the newly formed stratum corneum was partly parakeratotic (Fig. 1e). Epidermal cells started to migrate on the first culture days, forming a multilayered, short epibolus (Fig. 1c), whose most superficial cells were cornified.

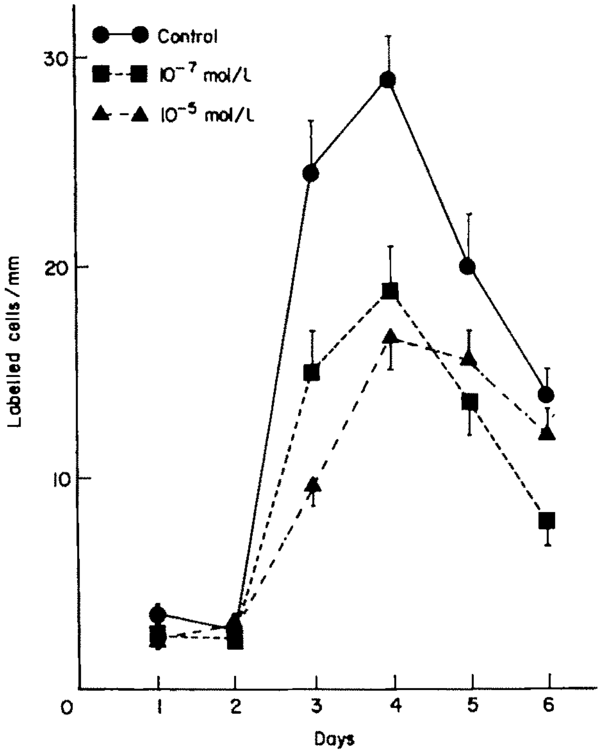

FIGURE 2. Effects of hydrocortisone on the number of labelled nuclei in the epidermis per 1 mm of epidermis per hour. Means and standard errors of the means were calculated from three experiments, each including six explants per time point.

Few epidermal cells were labelled with ^{3}H-thymidine on the first and second days of the culture (Fig. 2). The number of DNA-synthesizing cells was increased on the following two days (days 3 and 4) and declined thereafter. This increase of cells in S-phase, which most likely leads to a higher rate of cell proliferation, may account for the thickening of epidermis seen 2–4 days later.

Epidermis tended to be thinner in explants cultured with 10^{-5} and 10^{-7} mol/l hydrocortisone (Fig. 1a). The thinning was due mainly to fewer cell layers (Fig. 1b), and the mean cell height was not significantly reduced by the hormone (Fig. 1a). Hydrocortisone (10^{-7} and 10^{-5} mol/l) caused a significant decrease in the number of DNA-synthesizing cells in epidermis ($P < 0{\cdot}05$ for both hormone concentrations when calculated over the entire culture period; Fig. 2). This effect was most prominent on the third and fourth culture days, and the higher hormone concentration was more potent than the lower (Fig. 2). Hydrocortisone slightly, but not significantly, enhanced the migration of epidermal cells (Fig. 1c).

Besides the inhibitory action on epidermal cell renewal, hydrocortisone appeared to normalize the keratinization of cultured epidermis. The hormone increased the relative volume of orthokeratotic stratum corneum (Fig. 1e) and the number of granular cells (Fig. 1f). The lower concentration of the hormone (10^{-7} mol/l) appeared to be even more potent in these respects than the higher concentration ($P < 0{\cdot}001$ for granular cell number). When, instead, the hormone concentration was decreased to 10^{-9} mol/l the hormonal effects did not reach any more statistical significance.

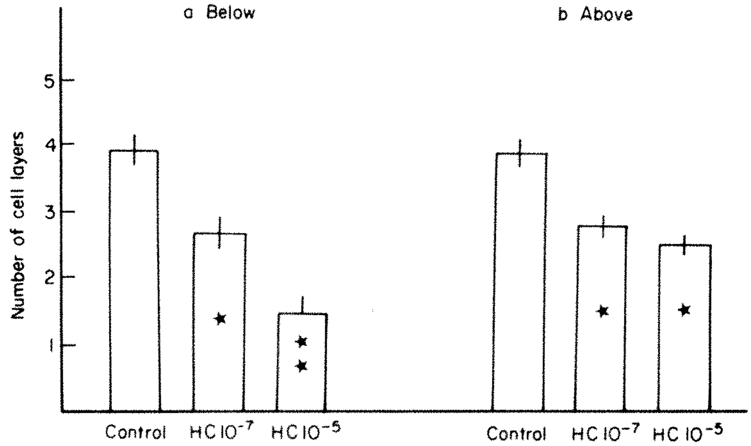

FIGURE 3. Effect of hydrocortisone on the transit time of epidermal cells in culture. Mean number of vital cells layers (± s.e.mean) (a) below and (b) above the labelled cell on the seventh culture day (6 days after the ^{3}H-thymidine pulse). The significances of differences are indicated as in Fig. 1.

Epidermal cells labelled with ^{3}H-thymidine on the first culture day were shown to move from the basal layer to the stratum corneum. The transit time of the epidermal cells from the basal layer to the granular layer was 6 days both in control and hormone treated cultures. It appeared thus not to be controlled by hydrocortisone in the cultured skin. Fewer cell layers were, however, observed below the labelled cells in hormone treated cultures than in control cultures (Fig. 3a), indicating the antimitotic action of the hormone. There were also less cell layers above the labelled cells in the hydrocortisone treated cultures on the fifth and seventh culture day (Fig. 3b), suggesting facilitated cornification within the first days in culture.

DISCUSSION

Hydrocortisone in physiological concentration decreased the labelling of stimulated epidermal cells by ^{3}H-thymidine. In contrast to earlier findings (Delforno, Holt & Marks, 1978) the mean cell height in epidermis was not significantly affected by hydrocortisone in the present study.

Assuming that the labelling of epidermal cells accurately measures not only DNA synthesis but also the mitotic rate, this antimitotic action could account for the thinning of epidermis by hydrocortisone. The antiproliferative action of hydrocortisone was most apparent during the active phase of proliferation *in vitro*, i.e. on the third and fourth days of culture. This agrees well with an earlier report about the dependence of glucocorticoid effects on the basal mitotic rate of the tissue (Marks *et al.*, 1973).

Despite the antiproliferative action of the hormone on epidermis the migration of epidermal cells was virtually unchanged in the presence of hydrocortisone. This confirms an earlier assumption that epidermal cell migration and the rate of epidermal cell proliferation are not closely connected (Devitt *et al.*, 1978).

The present study supports the idea that hydrocortisone in physiological concentrations is advantageous (Fell, 1962; Sugimoto & Endo, 1969) or even essential for epidermal differentiation. Hydrocortisone normalized the keratinization process as indicated by the increased number of granular cells and the more orthokeratotic stratum corneum. The transit time of epidermal cells *in vitro* appeared to be 6 days, which is considerably shorter than that of normal epidermis *in vivo* (Frost, Weinstein & van Scott, 1966). Although the hormone appeared not to have any effect on the transit time of epidermal cells from the basal layer to the granular layer, it may have facilitated the cornification during the first few days of culture. Thus hydrocortisone causes alterations both in cell proliferation and differentiation. On the basis of the present findings it was not possible to separate the relationship between proliferation and differentiation (Fry, Almeyda & McMinn, 1970; Goodwin, Hamilton & Fry, 1973). Both stimulation of proliferation and defective keratinization (parakeratosis) occurred in control cultures. It is a common concept that a defective keratinization is a result of enhanced proliferation in hyperproliferative skin diseases like psoriasis. However, it has been reported that maximally stimulated epidermopoesis in normal epidermis does not result in parakeratosis unless there is a defect in cell differentiation (Christophers & Braun-Falco, 1970). The growth inhibition and enhancement of keratinization in the present study did not seem to be closely interconnected since the lower concentration of the hormone, which was less potent in inhibiting proliferation, was more effective in enhancing the differentiation.

Many experimental models have been proposed both in man and in animals in order to study the effects of regulatory substances on epidermal regeneration or to predict the efficacy and potential side effects of different corticosteroids. Many of them are impractical and the clinical relevance of the results obtained difficult to evaluate. Human skin culture may offer some advantages. It makes possible the direct study of the antiproliferative and atrophogenic effects of individual compounds without interference from systemic factors. An accurate ranking of test compounds with regard to potency is possible under well defined and repeatable *in vitro* conditions and, needless to say, the results are applicable to normal human skin.

ACKNOWLEDGMENT

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The rate of corneocyte formation in microscopic lesions in patients with active psoriasis

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SUMMARY

Microscopic lesions in active psoriasis were identified after careful examination with a magnifying glass in areas free of macroscopic lesions and of hair follicles. Biopsies were excised without anesthesia, immediately frozen and cryostat-sectioned. Care was taken to preserve the horny layer which was stained to reveal corneocyte membranes and nuclei when present. The interlocking sheets of corneocyte layers made it feasible to outline and trace intercellular spaces parallel to the surface and count the number of corneocyte layers between pairs of these. It was assumed that the location of such a space in the horny layer indicates the period of time from its formation. This information was used to assess the rate of corneocyte layer formation at specified intervals of time. The result shows that adjacent areas of the horny layer could be compared and relative rates of corneocyte layer formation computed.

In the parakeratotic part of the early lesions of psoriasis studied the rate of corneocyte layer formation was 15 times higher than that in the non-involved skin. The peak rate, coincident with parakeratosis, was preceded by a number of thickened corneocyte layers. These also showed an increased rate of formation, up to 6·6 times that in non-involved skin. There was no acanthosis in the lesions studied. A subepidermal cellular infiltrate was observed. Periods of rapid corneocyte formation with parakeratosis were often short (not more than one day). These were interrupted by periods with a lower rate. The relationship between corneocyte morphology and stratum corneum kinetics is a distinct feature in the early psoriatic lesion.

The epidermal turnover consists of the production of new cells in the germinative layers followed by their migration up to the surface and exfoliation. When cell production is increased, the Malpighian layer may be enlarged or corneocyte formation enhanced, or both these effects may be present simultaneously. In psoriasis cell production is exaggerated. Various methods have been used to measure this process, including ^{3}H-thymidine autoradiography, measure-

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ment of the mitotic index, and cytophotometric techniques (Weinstein & Frost, 1967; Van Scott & Ekel, 1963; Bauer & De Grood, 1975).

The transit time of cells in psoriatic epidermis has been estimated from the time taken for labelled germinative cells to appear in the superficial part of the Malpighian layer (Rothberg, Crounse & Lee, 1961; Weinstein & Van Scott, 1965). The production of corneocytes is the net result of germinative cell proliferation. The rate of cell transit has been estimated by staining the horny layer with a fluorochrome and studying its time of disappearance or measuring the formation of new layers underneath the stained portion of the horny layer (Hammar, 1973; Johannesson & Hammar, 1980).

The present study is based on the following principles. It is well established that the horny layer consists of corneocytes glued together by an intercellular cement (Elias *et al.*, 1979). The intercellular spaces between flattened corneocyte surfaces are oriented parallel to the skin surface and to the underlying granular layer.

Assume that a space is traced through a portion of the horny layer. The apposition of newly formed corneocytes at the bottom of the horny layer will move this space towards the surface, and in time another space will be formed beneath the space being followed. Thus, the position held by an intercellular space within the horny layer is a coordinate of time. The most superficially located spaces are those that are formed earliest. Thus the first principle is that a specified location of a space within the horny layer is an indication of the time of its formation.

Assume, further, that in a study of the horny layer two spaces parallel to each other are traced and outlined. In accordance with the first principle this means that a time interval has been defined. The number of corneocyte layers between these two spaces is counted in two specific areas. If the same number is found at the two locations, this means that the layers formed in the interval are the same, i.e. the rate of corneocyte formation has been the same at both sites. This gives the second principle, that the number of corneocyte layers between two specific intercellular spaces can be used as a measure of velocity (layers formed per time interval). Thus, a finding of a different number of corneocyte layers between the two spaces at nearby positions within the horny layer means that the rates of corneocyte formation at these locations have differed. This paper describes the application of these principles to a study of the early lesion of psoriasis.

MATERIALS AND METHODS

Patients

Seven patients supplied twenty biopsies for evaluation of the method and the present results are based on seventeen further biopsies from ten patients. The patients were 33–44 years old. Eight were males. All had guttate or nummular psoriasis. The lesions were disseminated, untreated and at an acute stage. The thigh skin was carefully examined under a magnifying glass, × 3–10, for detection of minute lesions located at a distance from the visible lesions and clearly within the interfollicular fields. Without anaesthesia a punch biopsy 1–2 mm thick and 2–3 mm in diameter was excised, immediately frozen on solid carbon dioxide with the dermal side down, transported in a cold environment and kept in a deep-freezer at −90°C until sectioned.

Sectioning

Cryostat sections 6 μm thick were cut. They were attached to the slides by application of mild heat, 30°C for 2 days. Various stains could then be applied and withdrawn without any alterations in the morphology of the section.

Staining

Staining was done by application of 38 mM fluoroscein isothiocyanate (Sigma, St Louis) in water (Christophers, 1970) for 2 min, followed by two washes in 67 mM KCl. The nuclei in the parakeratotic horny layer and in the rest of the section stained satisfactorily with a solution consisting of 3·4 mM 4-4-diamidinodiphenylamine dihydrochloride (May and Baker 938) as described by Tring & Murgatroyd (1976), and 0·15 M NaCl in water applied for 10 s, followed by one wash in 67 mM KCl. The section was destained with 0·6 M acetic acid-0·15 M NaCl solution and covered with a coverslip. After reading, the coverslip was removed and the section was fixed in 70% ethanol, stained in haematoxylin and eosin and mounted permanently.

Readings

The sections were examined and photographed in a Zeiss Universal microscope in unfiltered UV light and with phase contrast at × 64, 100 and 500. A Nikon camera and Kodak Panatomic-x film, ASA 32, were used. Both UV and phase contrast micrographs were prepared and readings were made from enlargements of the negatives. Care was taken to exclude material in which a perifollicular lesion or a sweat duct in the lesion occurred. A representative lesion and its vicinity are depicted in Figs 1–3. Lines of examination perpendicular to the surface (A–F, Fig. 2) were selected at intervals from the normal end to the central part of the lesion. In the normal part of the horny layer (line F, Fig. 2) an intercellular space located just above stratum granulosum was chosen. From the enlargement a diagram was drawn depicting the pathway of this space throughout the section and into the centre of the lesion. At each point where a line of examination crossed the space tracing the number of corneocytes hit by this line was counted from the border of the stratum Malpighii up to the indicated point. At a few corneocyte layers higher up along the reference line F, a new space was followed and corneocytes were counted as

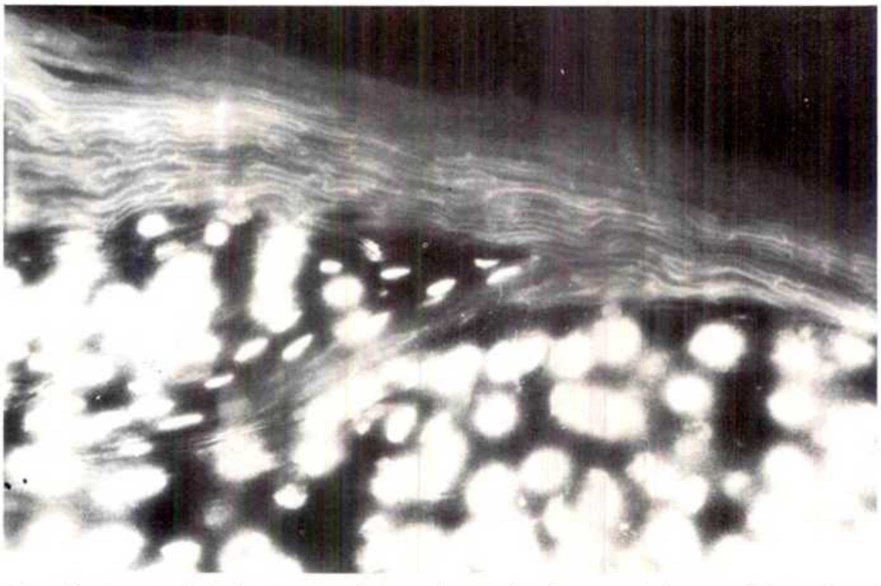

FIGURE 1. A microscopic lesion in a psoriatic patient and evaluation of the rate of formation of the corneocyte layers. Cell boundaries are stained by fluorescein isothiocyanate and nuclei by May & Baker 938 (× 840).

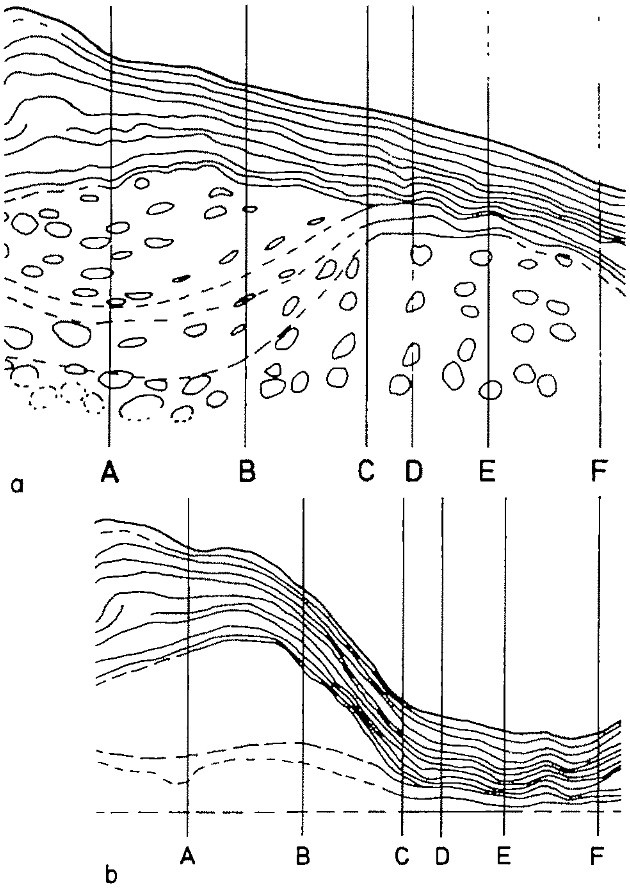

FIGURE 2(a) and (b). Drawings of the lesion in Fig. 1 with the examination lines A–F indicated. Line F is the reference. At five places along line F, intercellular spaces were selected and followed towards the centre of the lesion. At each point of intersection with the examination lines, the number of corneocyte layers interposed between a selected space and the next space was counted.

previously. In this way a set of intercellular spaces parallel to the surface was identified and between them the number of corneocyte layers was counted at the predetermined points. A particular intercellular space was usually easy to identify throughout the horny layer. In parakeratotic regions both the UV and the phase contrast images had to be used for proper identification of the space. The way in which a particular space was followed is exemplified in Fig. 4.

Inflammatory cells were counted in parakeratotic regions of the horny layer in the H & E slides. The nuclei of these cells were used to discriminate them from the flattened ellipsoid parakeratotic corneocyte nuclei.

Calculation

Rate of corneocyte formation. The normal horny layer served as a reference for the

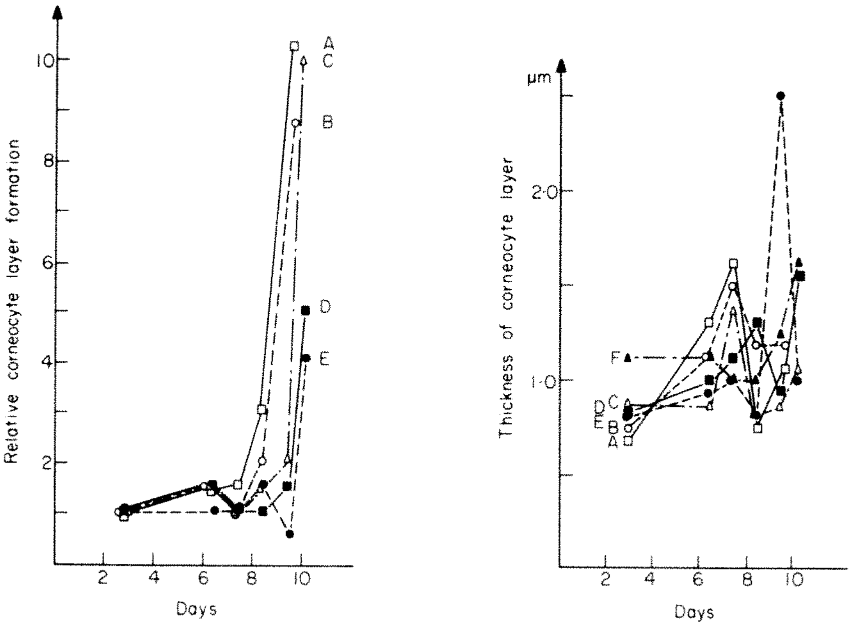

FIGURE 3. (a) The rate of corneocyte layer formation at the five examination lines A–E relative to the reference at line F is given on the ordinate (values from Table 1). The mean rate of replacement in the non-involved skin, two layers per day, was used to convert numbers of layers interposed between two successive examination points into a measure of time, which is given on the abscissa. Zero time represented the top layer, which was formed 10·5 days prior to the supragranular layer of corneocytes. (b) Corneocyte thickness in relation to the time of its formation. Zero time represents the same as in Fig. 3a. The thickness of the corneocyte layers between two successive examination points was measured and the average thicknesses recorded for lines A–F are given.

The graphs show thickening of the corneocyte layers on the 7th day (A–C) preceding the burst in the rate of layer formation on the 9th–10th day, starting from the centre (A) and spreading out towards E.

determinations. Within each pair of selected neighbouring intercellular spaces the counted numbers of corneocyte layers gave the rate of corneocyte formation for the time interval indicated by the position of the particular spaces. The relative rate of corneocyte layer formation at a specified examination line was calculated by computing the ratio between the number of corneocyte layers at this line (e.g. lines A–E in Fig. 2) and the number at the reference line (e.g. line F in Fig. 2). It has been found that in the non-involved skin 1·8 corneocyte layers per day (Hammar, 1973) or 2·2 layers per day (Johannesson & Hammar, 1980) are replaced. The mean of two layers per day was taken as a basis for converting the number of layers at the reference line to a time measure (Fig. 3). Twenty-one layers were counted in total corresponding to 10·5 days from the formation of the top layer to the bottom layer. Using the time axis, the events in this time interval can be depicted as seen in Fig. 3.

Point estimates. The rate of corneocyte formation was estimated by our method at two junctions:

(1) Between nucleated corneocytes and thickened non-nucleated cells (width $> 1{\cdot}0$ μm).

(2) Between thickened and normal corneocytes (width $< 0{\cdot}9$ μm).

All measurements were made either along a special examination line or along a specified

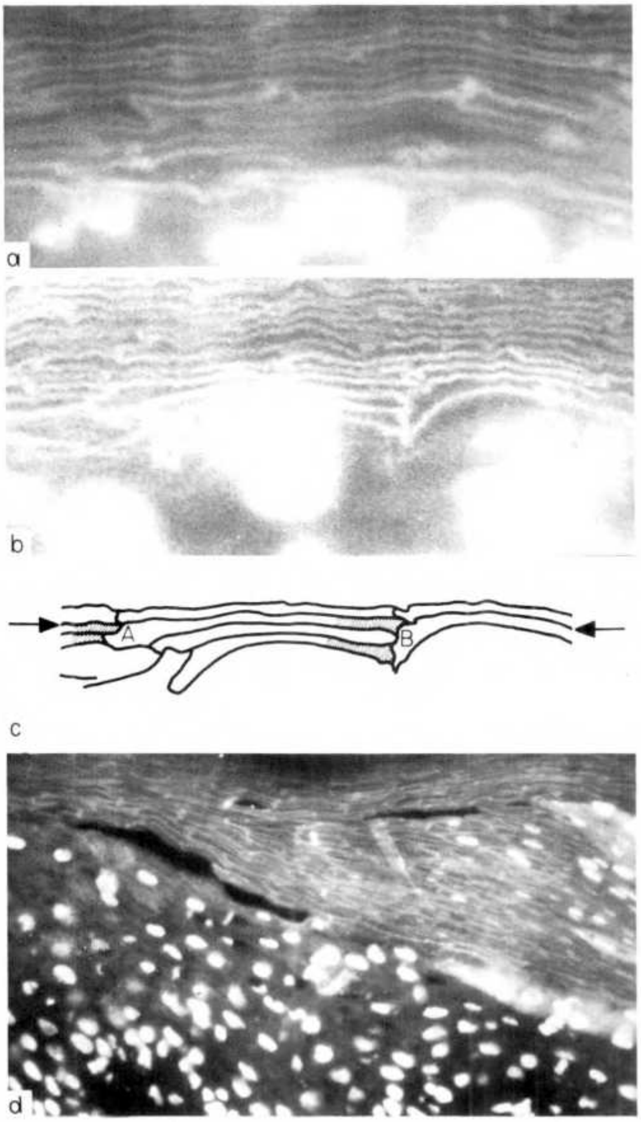

FIGURE 4. Fluorescein isothiocyanate stained horny layers. (a) The horny layer at the border of a microscopic lesion displays laminar intercellular spaces between corneocytes which are easy to follow, as they run parallel to the surface (× 1630). (b) Appearance of unequal numbers of corneocytes adjoining each other. The intercellular space to be followed may be based on the fact that the space above or the space above that is continuous (× 1630). (c) Schematic drawing of a section from (b). Two or more corneocytes adjoin the heads designated by A and B. The arrows point to a space that is to be followed. (d) Border of a parakeratotic lesion. Individual cells are sufficiently well outlined to permit reproducible tracing to be made of intercellular spaces through the area and to allow the number of cells between them to be counted (× 260).

It is assumed that parallel intercellular spaces, as in (a) indicate a constant rate of corneocyte layer formation throughout the period during which the present horny layer was developed. In (d) the superficial part of the horny layer shows parallel spaces. In the bottom part several more corneocyte layers are interposed between the top part and the Malpighian layer at the right-hand side of the section than at the left-hand side. The greater interposition of layers to the right means that the rate of corneocyte layer formation has differed considerably in the two parts of the epidermis. The detail in (b) shows how such a difference arises.

TABLE 1. Rate of corneocyte layer formation in a microscopic psoriatic lesion (see Fig. 1). The number of corneocyte layers was counted between a number of specified intercellular spaces at various examination lines. A corneocyte stratum is defined as being located between a consecutive pair of intercellular spaces. The numbers of layers relative to those at the reference line F are shown. Since each of the spaces traced through the horny layer is assumed to be formed at the same time, these values simultaneously give the rate of formation relative to that of the reference rate. Assuming that two layers are formed per day, the time basis of these changes is also obtained. The distance between examination lines is indicated at the bottom of the table. A high rate of corneocyte formation coincided with parakeratosis but was preceded by an aggregation of structurally altered corneocytes

Corneocyte stratum (location)	Relative rate of corneocyte layer formation Examination lines						Reference line F	
	Centre A	B	C	D	E	Periphery F	Layers per stratum (No.)	Cumulated age (Days)
Superficial	1·0	1·0	1·0	1·0	1·0	1·0	12	6
	1·5	1·5	1·5	1·5	1·0	1·0	2	7
	1·5*	1·0*	1·0*	1·0	1·0	1·0	2	8
	3·0*	2·0*	1·5*	1·0	1·5	1·0	2	9
Supragranular	10·2†	8·7†	2·0	1·5	0·5	1·0	2	10
			10·0†	5·0*	4·0*	1·0	1	10·5
Distance (μm)	50	47	20	28	44			

* Thickened corneocytes. † Nucleated corneocytes present.

intercellular space as exemplified in Table 1. The rate at junction (1) was taken as the average of 10·2 and 3·0, i.e. 6·6, along line A, or as the average along the bottom space of 10·0 at C and of 5·0 at D (7·5). If thickened corneocytes were present underneath a nucleated portion along the centre line A, a third average was also computed. The three averages then constituted junctions at the top, the side and the bottom of the nucleated corneocyte portion. The rate at junction (2) was obtained in the same manner for the thickened and adjacent normal corneocytes at the top and the side junctions.

Variance of point estimates was obtained by measuring the same slides at an interval of one to three months in four patients, all of whom had two lesions examined. The values for the rate of corneocyte formation at the junctions of normal and thickened cells, and of thickened and parakeratotic cells, and those for the maximal rate were submitted to analysis of variance. The errors of the three measurements were 13, 12 and 11%, respectively, given as coefficients of variation of the mean rates.

Corneocyte diameter. The corneocyte cell diameter was estimated from the lengths of adjoining cells parallel to the surface. In order to reduce a possible systematic error due to the sectional plane through the horny layer, groups of at least six adjoining cells in each of more than three apposite layers were counted. The four top and bottom layers of the stratum corneum were excluded. In one lesion the count was made for the parakeratotic, the thickened and the normal

TABLE 2. The relative rate of formation of corneocyte layers in two lesions in each of four patients. The slides were examined on two occasions with an interval of 1–3 months. The three points of measurement are the same as are depicted in Fig. 6. Interaction was significant in two of the measured groups, with P near to 0·05, indicating variation due to the different locations of individual lines of examination on the two occasions of measurement rather than differences in mean values of the two lesions at the two points in time

			Junction between			
	Maximal (lesions)		Parakeratotic and thickened cells (lesions)		Thickened and normal cells (lesions)	
Patient	1	2	1	2	1	2
1	13·0, 13·5*	17·5, 17·0	6·1, 7·6	7·0, 7·8	2·0, 1·4	2·4, 2·5
2	10·5, 11·0	11·3, 12·3	5·9, 5·4	6·3, 6·9	1·8, 2·0	2·7, 2·7
3	14·5, 16·0	13·5, 16·5	7·9, 8·2	7·8, 10·2	2·8, 2·5	3·3, 2·4
4	7·5, 9·0	12·5, 16·5	5·9, 6·3	5·7, 6·8	3·0, 2·5	2·0, 2·3
Analyses of variance (degrees of freedom, mean squares)						
Patient	3	9·95‡	3	2·52†	3	0·16
Lesions	1	15·26‡	1	0·85	1	0·17
Interaction	3	4·28†	3	0·07	3	0·21†
Error	8	0·98	8	0·33	8	0·05

* Rates obtained from the first and second examination, respectively.

† $P < 0·05$.

‡ $P < 0·01$ according to F-test against the error.

corneocytes, respectively. The mean diameter of the corneocytes (D) was estimated from apparent lengths of the sectioned cells (L):

$$D = \frac{4}{\pi}\cdot\frac{1}{n}\Sigma L$$

where n is the number of measured cells. This value was obtained from the expected length, E(L), when the sectional plane through assumed circular corneocytes was random. If the angle *in origo* between the bisector of the plane and the intersection of the plane with the circumference is α, then:

$$E(L) = D\int_{0}^{\frac{\pi}{2}}\sin^2\alpha d\alpha = \frac{\pi}{4}D$$

The relationship between the variance of D (V_D) and the variance of L (V_L) is found by a similar procedure to be:

$$V_D = \left(\frac{32}{3\pi^2} - 1\right)V_L$$

Acanthotic index. The index was defined as the ratio of the perpendicular length of the Malpighian layer overlying a rete ridge to that of the adjacent epidermis above a dermal papilla. It was used to describe the epidermis at the reference point.

RESULTS

Morphology

In the seventeen microscopic psoriatic lesions examined the epidermis was increased in thickness but no dermal papillomatosis was present. In the centre of the lesion one or several islets of parakeratotic cells were present in the horny layer. In between, an ordered structure of apparently normal or thickened corneocyte layers were seen.

The central nucleated portion of the horny layer lesions had a surface length of about 400 μm (range 200–800 μm) and its mean total length was almost 600 μm. The reference line (e.g. F, Fig. 2) was located 250 ± 80 μm (s.d.), on the average outside the lesion. The acanthotic index at the reference line was 1·39 ± 0·16 (s.d.).

Near a parakeratotic lesion, two changes in the morphology of the horny layer were seen: (1) The thickness of a corneocyte could be increased to 3–5 times that of its neighbour. (2) One cell—which could be thin or thick—adjoined up to four cells on one side but only one cell on the other (Fig. 4b–d). The corneocyte cell diameter, estimated from the corneocyte lengths given in Fig. 5, was decreased from 40·7 ± 0·1 μm (s.e. mean) in the normal horny layer to 34·2 ± 0·2 μm (s.e. mean) and 36·5 ± 0·2 μm (s.e. mean) in lesional areas with thickened and parakeratotic cells, respectively. The diameters in the lesional parts, 16% less than the normal, were significantly smaller ($P < 0{\cdot}001$).

Rarely, inflammatory cells were aggregated within the epidermis or in the parakeratotic islets. Analysis of each section of the horny layer showed that in five lesions there were no inflammatory cells and that in another three lesions there was a questionable one. One inflammatory cell was found in five lesions and two or more in the remaining four lesions. An

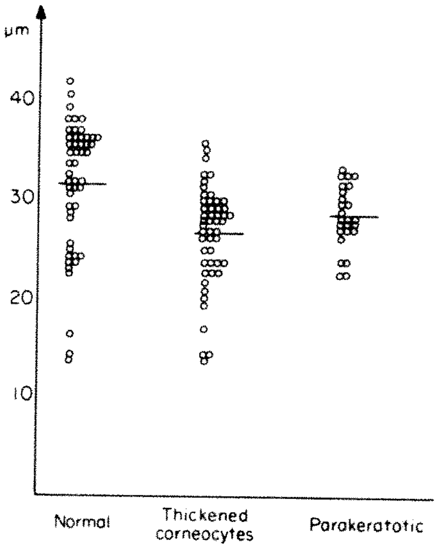

FIGURE 5. Apparent lengths of corneocytes in the stratum corneum in one patient. The distribution is skew in the two left-hand groups, as expected (see text). The parakeratotic counts were limited and lacked a tail, since small-sized cells would appear non-nucleated. Since the peak coincides with that of the thickened cells, these two groups probably show the same distributions. The mean length in the non-involved area was 31·7 μm, corresponding to a diameter of 40·4 μm. The corresponding values for the thickened cells were 26·9 and 34·2 μm, respectively. The diameter was estimated from apparent lengths as described in the text.

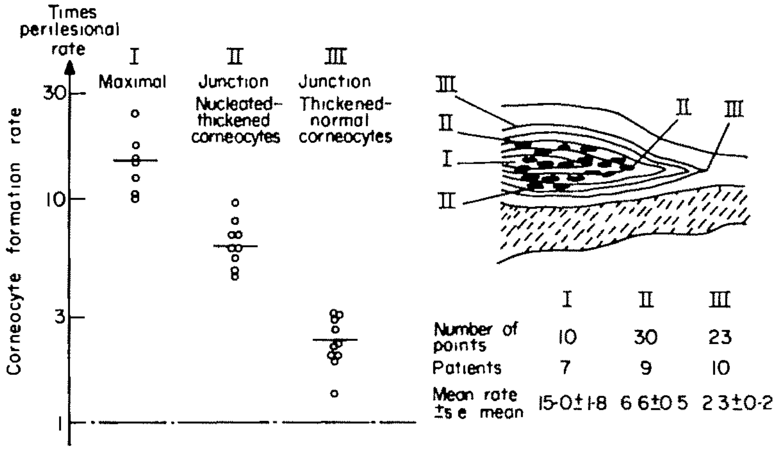

FIGURE 6. Rates of corneocyte layer formation in different areas within psoriatic lesions in relation to the rate in the adjacent non-involved apparently normal horny layer. The rate at the two junctions was taken as the average between rates nearest to the point in question. Since the rates did not differ from each other at the top, the side or the bottom (when encountered) of the lesion, these were averaged for each patient. A logarithmic ordinate was used for the rate in order to indicate a proportional increase between the groups.

inflammatory infiltrate was always present in the dermis. Generally, these cells were most abundant in regions beneath the parakeratotic islets.

The rate of corneocyte formation

In the microscopic lesion summarized in Figs 1–3 and Table 1 an increased thickness of the corneocytes was evident two days prior to a sharp rise in the rate of corneocyte formation. This result was also the mean in five patients.

In seven lesions in five patients the nucleated cell nest was surrounded by thickened non-nucleated corneocytes. The maximal time period during which the nucleated cell portion was formed was 1·5 days, with an average of only 0·5 days.

The observed rates of corneocyte formation are summarized in Fig. 6. The mean maximal rate was 15·0 ± 1·8 (s.e. mean) times that of the reference level. This occurred only in an area with heavy accumulation of nucleated corneocytes. The rates at the junction between nucleated and thickened cells at the top, the side and the bottom of the parakeratotic islet were not different from each other at the thirty measurement points. The mean rate at these junctions was 6·6 ± 0·5 (s.e. mean) times that of the reference level. The rate at the junction between thickened and normal corneocytes was also the same at the top and the side of the lesion at twenty-three measurement points; here the mean rate was 2·3 ± 0·2 (s.e. mean).

The natural progress of the lesions is depicted in Fig. 7 and Fig. 1. In Fig. 7a the parakeratotic cells are seen to involve a large proportion of the epidermis, leaving only a few cell layers intact in its basal part. There is a columnar arrangement of cell nuclei in the epidermis. The maximal rate of corneocyte formation in this lesion was twenty-four times that in the adjacent normal skin. In the section in Fig. 1 the rate of corneocyte formation as recorded just above the Malpighian layer is slowed down by the appearance of non-nucleated cells underneath the parakeratotic portion. The thin Malpighian layer in Fig 7a is not present any longer when a parakeratotic islet has appeared, see Fig. 1. Acanthosis occurs later (Fig. 7c). This pulsatile rate of corneocyte formation may be repeated by successive new bouts of parakeratosis as seen in Fig. 7c. Between

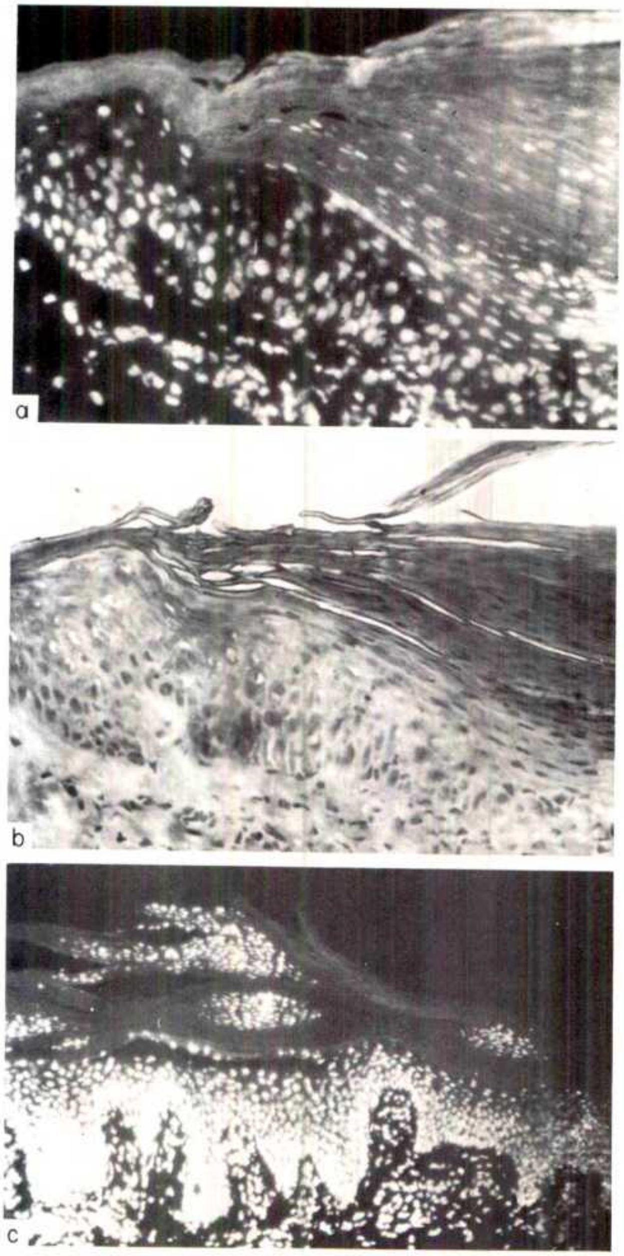

FIGURE 7. (a) Cryostat section of a microscopic psoriatic lesion stained as in Fig. 1. The parakeratotic horny layer occupies a greater portion of the Malpighian layer than does the non-involved left part of the epidermis. There is a columnar array of nuclei. A cellular infiltrate is seen in the upper dermis (× 195). (b) The same section as in (a) restained with H & E. Parakeratotic layers are extended deep into the Malpighian layer. Only elongated nuclei are seen in the horny layer (× 195). (c) Section of an older lesion stained as in (a). Groups of parakeratotic islets surrounded by thickened non-nucleated corneocytes are present. Beneath these areas two new parakeratotic lesions are being formed; to the left there is parakeratosis, and to the right the nuclei of the superficial stratum Malpighii are spaced and show a columnar array (arrows) (× 80).

the parakeratotic portions in this lesion the rates were between two and five times that in the adjacent non-involved skin, and in its centre a rate of up to fifteen times the latter rate was noted.

DISCUSSION

The method

The described method is new in kinetics applied to the skin. Its advantages lie in the microscopic discrimination of neighbouring areas, in the possibility of distinguishing between their rates of corneocyte formation, and in the recorded information for the time period during which the horny layer under investigation was formed. Distortion is within the limits of that due to preparation for histology. The possibility of activation of the skin by a preceding trauma does not have to be considered, as in the case of injection of a tracer, for example.

Furthermore, the sections may be used for other purposes after completion of the kinetic measurement, e.g. for investigation of immunological deposits or of types of inflammatory cells present, or analysis of the epidermis itself by histological techniques. This will be the object of a future study. The uncertainty of the method may lie in the degree of accuracy of tracing an intercellular space and the validity of converting sequential intercellular spaces to discrete time intervals. Morphological evidence of the validity of the method is given in the results. The irregular stacking characteristics of corneocytes in the horny layer in humans have been described by Mackenzie (1975) and Christophers (1970). This was also our finding, which meant that tracing of a space was not difficult (Fig. 4). Supporting the validity is the observation after staining with dansyl chloride (Johannesson & Hammar, 1978), when the fluorescent dye stained the complete horny layer, the new unstained layers formed underneath were arranged in strata.

Addition of salt to the dye solutions increased the stainability. No sodium hydroxide solution step (Christophers, 1970) was included to swell the corneocytes. However, our procedure did make the cells in the normal horny layer swell to some extent. The thickness recorded was about four times that reported by Holbrook and Odland (1974). The cohesion and ordered structure of the horny layer was undisturbed, however, possibly owing to the completeness of the swelling, since no rupturing of cell boundaries was seen.

The microscopic lesions in psoriasis

The structural alteration of the corneocytes in disease was pointed out by Menton and Eisen (1971). Braun-Falco (1971) examined the perilesional skin of psoriasis and remarked upon the histochemical parakeratosis in the normal-looking parts of the horny layer. The thickened corneocytes observed around areas of parakeratosis may well correspond to the histochemical parakeratosis of Braun-Falco (1971). Furthermore, a decrease of 16% in the diameter of thickened and parakeratotic corneocytes was found (Fig. 5), which is similar to that recently reported by Goldschmidt (1979).

It is improbable that these thickened cells were artifacts due to the sectioning, since in all parts of a given section such cells could be found adjoining normal-sized corneocytes.

The observations depicted in Fig. 7a,b, Fig. 1 and Fig. 7c, showing the temporal aspect of an evolving psoriatic lesion, were regular and constant. This indicates that after the initiation of psoriatic reaction the epidermis sheds all the differentiated Malpighian cells into a parakeratotic horny islet leaving only one or a few basal layers. In these portions of the epidermis regular stacking of cell nuclei was seen. These were often retained also in the parakeratotic islets (Fig. 7a,c).

The inflammatory reaction in the lesions has been underlined by Braun-Falco (1971, 1977) and Chowaniec & Jablonska (1979).

The very early lesions studied in this investigation displayed a dermal cell infiltrate in all cases, but more rarely there was aggregation of inflammatory cells in the parakeratotic region of the horny layer. This finding suggests that the major target of the infiltrate must reside in the basal part of the epidermis. If this infiltrate is important for the change into parakeratosis, it might be expected that there would be more of these cells among the parakeratotic corneocytes than were found (Fig. 7a,b).

Rate of corneocyte formation

The formation of the parakeratotic islet in the horny layer and its relation to shedding of a part of the Malpighian layer are findings in this study. Could the shedding be a necessary component of the observed increase in the rate of corneocyte formation? The following considerations would seem relevant in this context.

In the normal skin about forty-four keratinocytes are located under a corneocyte (Bergstresser & Taylor, 1977). In the microscopic psoriatic lesions of the present study, the Malpighian layer was not acanthotic (Figs 1, 4d and 7a). We may assume, therefore, that about thirty-two keratinocytes are located under the smaller thickened corneocytes (area 72% of normal) if the size of the keratinocytes in the Malpighian layer is not altered. About ten cells may be assumed to reside in the germinal part of the epithelium. This leaves us with twenty-two cells to be shed and transformed into small corneocytes of the parakeratotic islet. In the non-involved skin the rate of corneocyte formation was two layers per day. Shedding should then be associated with a rate of eleven layers per day relative to that of non-involved skin. The mean found was fifteen layers per day. The difference of four layers per day may well be due to the formation rate of thickened cells which appeared in advance of a parakeratotic islet (Table 1 and Fig. 6). A reasonable explanation might be that the short but high rate of corneocyte formation found in the present microscopic lesions represents additional shedding of a part of the epithelium to an otherwise increased rate of corneocyte formation. No consideration was paid to acanthotic psoriatic lesions, which may differ in many ways from those in the very early lesion investigated here.

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Enhanced monocyte and neutrophil cytotoxicity and normal cyclic nucleotide levels in severe psoriasis

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SUMMARY

Eighteen patients with active psoriasis were investigated for antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by monocytes and neutrophil leukocytes. Patients with extensive psoriatic lesions showed increased ADCC whereas patients with minimal psoriasis had normal monocyte and neutrophil function. After clinical remission the ADCC became normal. No stimulatory factors in psoriatic serum could be demonstrated. The increased monocyte and neutrophil cytotoxicity in severe psoriasis is not explained by altered cyclic nucleotide levels as cAMP and cGMP levels were normal in psoriatic monocytes and neutrophils showing both increased and normal ADCC. Our results indicate that increased ADCC is secondary to the psoriatic activity.

In the early lesion of psoriasis, leukocyte infiltrates are seen in the upper dermis when only minimal epidermal changes are observed (Braun-Falco & Christophers, 1974). Immunological abnormalities have recently been demonstrated in patients with psoriasis. These include a depressed cutaneous reactivity to streptokinase and streptodornase, to DNCB challenge and a decreased *in vitro* lymphocyte Con A responsiveness (Krueger, Hill & Jederberg, 1978a). Furthermore, enhanced neutrophil and monocyte chemotaxis and phagocytosis have been shown in psoriatic patients independent of the disease state (Krueger *et al.*, 1978b; Wahba *et al.*, 1978, 1979; Bar-Eli *et al.*, 1979).

Since agents that stimulate leukocyte cAMP depress chemotactic and phagocytic activity (Cox & Karnovsky, 1973; Rivkin, Rosenblatt & Becker, 1975), and as agents that increase cGMP enhance chemotaxis (Sandler, Gallin & Vaughan, 1975), it has been suggested that psoriatic leukocytes have depressed cAMP/cGMP ratios (Wahba *et al.*, 1978, 1979; Bar-Eli *et al.*, 1979) similar to the findings in proliferative epidermal lesions (Voorhees & Duell, 1971; Voorhees *et al.*, 1972, 1974).

This study demonstrates increased monocyte and neutrophil cytotoxicity only in patients with severe psoriasis. However, monocyte and neutrophil cyclic nucleotide levels were completely normal in these patients.

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MATERIALS AND METHODS

Subjects

Blood was drawn from eighteen psoriatic patients admitted to our department. Twelve of the patients were male and six female. The age range was 14–79 years, with an average of 49 years. The control group consisted of eight males and five females, aged from 22 to 57 years, with an average of 31 years. In addition to psoriasis, three had psoriatic arthritis. None of the patients had received systemic therapy for their psoriasis within one year before the investigation. The patients were treated with topical steroids and/or tar. The patient group was graded on a scale according to the extent of the psoriatic lesions. Grade 1—less than 10% of skin involved; grade 2—10–25%; grade 3—25–50%; grade 4—50–75%; grade 5—more than 75% of body surface. Eleven of the patients had more than 25% of the skin surface affected and seven had less than 25%. All patients were investigated during clinical exacerbation of their skin disease.

Isolation of monocytes and neutrophil leukocytes

Monocytes and neutrophils were isolated from heparinized venous blood as previously described (Borregaard *et al.*, 1979; Kragballe, Ellegaard & Herlin, 1980a). Of the mononuclear cells, 93% (89–97%) were monocytes as judged by non-specific esterase staining. The neutrophils were judged by morphological criteria and a purity of more than 95% was obtained.

Antibody-dependent cell-mediated cytotoxicity

The cytotoxicity assay was performed with minor modifications of the procedure previously described (Kragballe *et al.*, 1980b). The tests were set up in duplicates and all cells were resuspended in medium RPMI 1640 with 5% heat-inactivated fetal calf serum (FCS) and 100 iu/ml penicillin and 100 μg/ml streptomycin. For the monocyte ADCC human rhesus D-positive erythrocytes were used as target cells. Erythrocytes were labelled with sodium-51 chromate and sensitized with hyperimmune anti-D antiserum. The monocytes (100 μl of 1×10^6/ml) were mixed with 200 μl of pretreated erythrocytes (2 or 6×10^6/ml). As target cells for neutrophil ADCC assay human type A erythrocytes were employed. The neutrophils (100μl of 1×10^6/ml) were incubated with 100 μl of ^{51}chromium-labelled human type A erythrocytes (4 or 12×10^6/ml) and 100 μl of 40% hyperimmune anti-A antiserum.

The ADCC assay was initiated after centrifugation at 150 *g* for 1 min and the cells were incubated at 37°C for 1 h. Half of the supernatant (S) was then withdrawn and counted together with the residue (R) in a gammacounter. The chromium release was determined as follows:

$$\frac{2\times \text{S c.p.m.}}{\text{S c.p.m.}+\text{R c.p.m.}}$$

The specific cytotoxicity was obtained by subtracting the spontaneous release in tubes containing no phagocytes and the results were expressed as the number of target cells lysed per phagocyte.

In order to evaluate whether patient serum affects monocyte cytotoxicity, normal monocytes (4×10^6/ml) were preincubated with 25% serum from eight of the patients with increased cytotoxicity or with normal AB serum (fresh or frozen at −80°C). After incubation the cells were washed three times at 4°C in Hank's Balanced salt solution (HBSS). The cells were then adjusted to 1×10^6/ml and cytotoxicity was performed as described above.

Determination of cyclic nucleotides

Monocyte and neutrophil cAMP and cGMP were determined as previously described (Herlin, Petersen & Esmann, 1978) although with minor modifications. The cells were resuspended in HBSS with 2·5% FCS and adjusted to 15×10^6/ml. Triplicate samples of 100μl were transferred into polypropylene test tubes and 100 μl of boiling 0·25 M potassium phosphate buffer (pH 5·5) with 4mM theophylline, 9 mM 2-mercaptoethanol was added to each. After boiling for eight min the tubes were placed in an icewater bath and denatured protein was removed by centrifugation. From the supernatant 50 μl was removed for the cAMP assay and 100 μl for determination of cGMP. Cyclic AMP was determined by the competitive protein binding method and cGMP by the radioimmunoassay method using reagents from the Radiochemical Centre, Amersham, England. Cyclic AMP from stimulated cells was obtained similarly after incubation at 37°C for 5 min with solutions of either DL-isoproterenol-HCl, histamine dihydrochloride, or prostaglandin E_1 (PGE_1) (Sigma, St Louis, U.S.A.). Recovery of a cAMP standard solution (1 pmol/50 μl) was 98·5% ± 4·6% (mean ± s.e.m., n = 6) and of a cGMP solution (1 pmol/100 μl) 95·0% ± 2·8% (mean ± s.e.m., n = 6).

Statistical analysis

For statistical evaluation a Mann-Whitney test for two samples was used. A *P* value below 0·05 was considered significant. Spearman's coefficient of rank was applied in the correlation studies.

RESULTS

Monocyte and neutrophil cytotoxicity

The monocyte and neutrophil function was assessed by determining their ability to lyse antibody-coated human erythrocytes. In Table 1 it is shown that in psoriatics with more than 25% of the skin surface involved monocyte cytotoxicity was 131% ($P<0{\cdot}05$) (target/effector = 4:1) and 143% ($P<0{\cdot}01$) (target/effector = 12:1) of normal values. Patients with minimal or moderate skin lesions showed normal monocyte cytotoxicity.

Neutrophil cytotoxicity was investigated in ten of the eighteen patients. Cytotoxicity mediated by the neutrophils was only increased in severe psoriasis ($P<0{\cdot}02$) but only at high target cell ratios (Table 1).

TABLE 1. Antibody-dependent cell-mediated cytotoxicity in psoriasis

	Erythrocyte to monocyte ratio			Erythrocyte to neutrophil ratio		
	n	4 : 1	12 : 1	n	4 : 1	12 : 1
Healthy controls	13	1·23 ± 0·08	1·40 ± 0·09	8	0·58 ± 0·08	1·34 ± 0·09
Psoriatics:	18	1·40 ± 0·09	1·78 ± 0·11*	10	0·68 ± 0·11	1·54 ± 0·10
> 25% of skin involved	11	1·61 ± 0·11*	1·99 ± 0·13‡	6	0·82 ± 0·15	1·79 ± 0·10†
< 25% of skin involved	7	1·12 ± 0·06	1·44 ± 0·12	4	0·47 ± 0·08	1·24 ± 0·12

Values are expressed as the number of erythrocytes lysed per phagocyte (mean ± s.e.m.).
Incubation time 1 h.
P values versus controls: * $P<0{\cdot}05$; † $P<0{\cdot}02$; ‡ $P<0{\cdot}01$.

In Fig. 1 monocyte cytotoxicity is illustrated for patients divided into groups according to the extent of the disease. A significant correlation was found between the extent of psoriatic lesion and monocyte cytotoxicity ($r = 0{\cdot}7632$, $P<0{\cdot}01$). Three of the psoriatics also had psoriatic arthritis and the monocyte cytotoxicity obtained in these patients did not differ from ADCC in the other patients

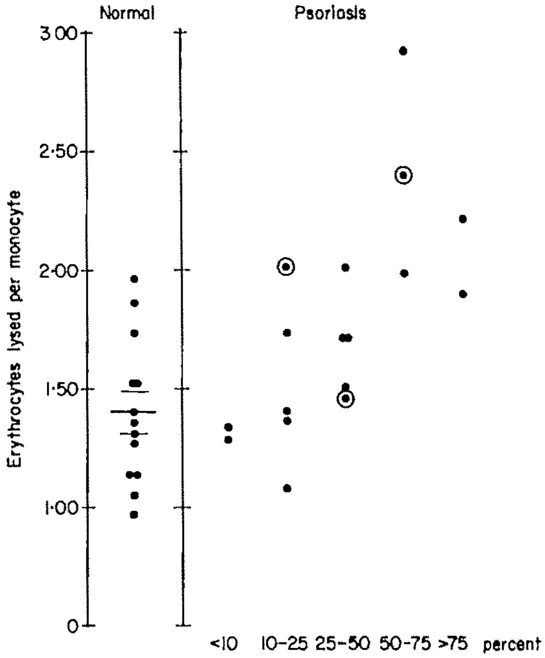

FIGURE 1. Monocyte ADCC in psoriasis in relation to percent of skin involved ($r = 0{\cdot}7632$, $P<0{\cdot}01$). Erythrocyte to monocyte ratio 12:1. Incubation time 1 h. Encircled dots represent patients with psoriatic arthritis.

with psoriasis. Between monocyte and neutrophil cytotoxicity a significant but not close relation was found ($r = 0{\cdot}6643$, $P<0{\cdot}05$, $n = 10$).

Influence of patient serum on monocyte cytotoxicity

In order to evaluate a possible presence of stimulatory factors in serum from psoriatic patients normal monocytes were preincubated with serum for 30 min at 37°C. In this condition neither AB serum nor serum from eight patients with increased monocyte cytotoxicity influenced cytotoxicity mediated by normal monocytes (Table 2).

TABLE 2. Effect of patient serum on normal monocyte cytotoxicity

	Erythrocyte to monocyte ratio	
Serum preincubated with	4 : 1	12 : 1
Normal AB serum (n = 8)	1·09±0·09	1·14±0·11
Patient serum (n = 8)	1·13±0·08	1·34±0·19
Monocyte cytotoxicity of patients with serum tested (n = 8)	1·46±0·06	2·10±0·15

Values are expressed as the number of erythrocytes lysed per monocyte (mean±s.e.m.). Incubation time 1 h.

Monocyte cytotoxicity after clinical remission

In four patients with severe psoriasis monocyte cytotoxicity was assessed 2–5 months after the first investigation. Three of these were in remission having less than 10% of skin surface involved. Monocyte cytotoxicity was within normal range in these patients (Fig. 2), but in one patient with still active and widespread psoriasis increased cytotoxicity was found.

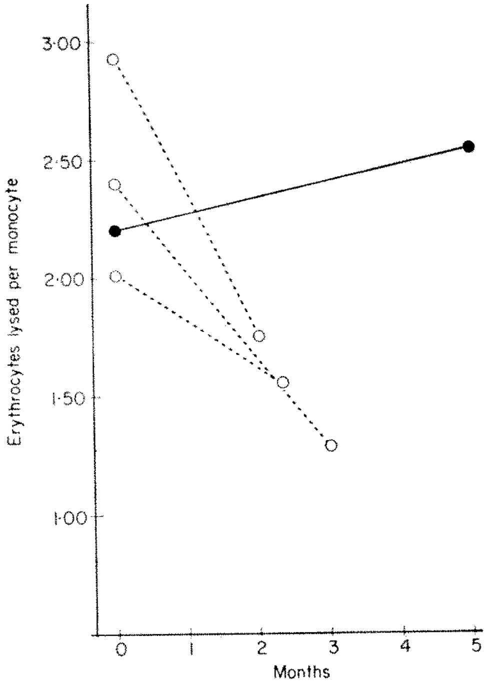

FIGURE 2. Monocyte ADCC in four patients with severe psoriasis. ○ - - - -○ : Patients underwent clinical remission; ●——●: no remission. Erythrocyte to monocyte ratio 12:1. Incubation time 1 h.

TABLE 3. Cyclic AMP in phagocytes from psoriatic patients and normals

	Psoriasis	Healthy controls
Monocytes	(n = 18)	(n = 13)
Buffer	24·1 ± 1·6	23·1 ± 1·2
Isoproterenol 10^{-5}M	61·6 ± 4·9	67·3 ± 6·8
10^{-6}M	55·0 ± 5·5	58·1 ± 9·1
Histamine 10^{-4}M	90·1 ± 10·5	91·9 ± 11·1
PGE$_1$ 3×10^{-5}M	164·1 ± 9·8	178·3 ± 17·4
Neutrophils	(n = 10)	(n = 8)
Buffer	12·7 ± 2·3	13·9 ± 3·2

Cyclic AMP is measured 5 min after addition of agents. The values are expressed as pmol cAMP per 10^7 cells (mean ± s.e.m.).

Cyclic nucleotide levels

Basal cAMP levels in highly purified monocytes and neutrophils from psoriatics were the same as in normals (Table 3). There was no difference in cAMP levels between patients with widespread or minimal dermal lesions (Fig. 3). When the monocytes were stimulated with isoproterenol, histamine, or PGE$_1$ normal cAMP values were obtained. Like the basal cAMP levels, the cAMP responses to stimulatory agents were not related to the severity of the disease. The cyclic GMP content was also normal in the unstimulated monocytes and neutrophils from the psoriatic patients (Table 4).

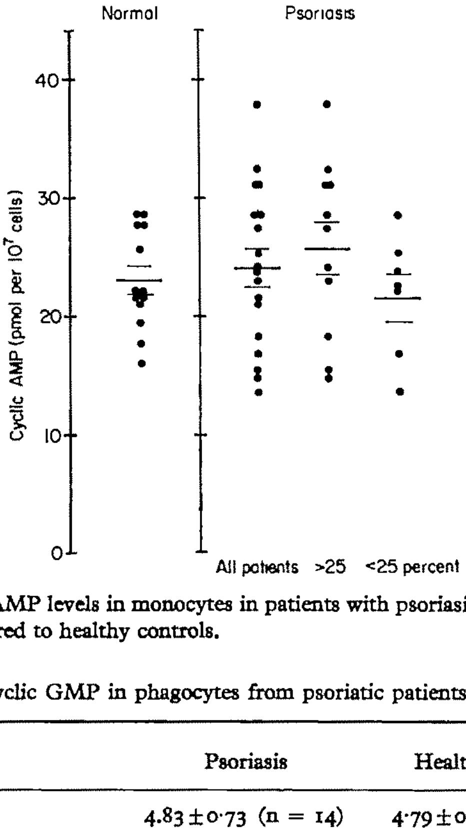

FIGURE 3. Basal cyclic AMP levels in monocytes in patients with psoriasis (more or less than 25% of skin involved) compared to healthy controls.

TABLE 4. Cyclic GMP in phagocytes from psoriatic patients and normals

	Psoriasis	Healthy controls
Monocytes	4.83 ± 0·73 (n = 14)	4·79 ± 0 85 (n = 10)
Neutrophils	4·26 ± 0·52 (n = 10)	5·05 ± 0·70 (n = 8)

Cyclic GMP values are obtained in unstimulated monocytes and neutrophils and are expressed as pmol per 10^7 cells (mean ± s.e.m.).

DISCUSSION

In psoriatic patients with extensive skin lesions we have demonstrated an increased monocyte and neutrophil cytotoxicity whereas normal cytotoxicity was found in patients with less than 25% of the skin involved. All patients were investigated during exacerbation of their psoriasis and there was a significant correlation ($P < 0{\cdot}01$) between increase in cytotoxicity and the extent of the epidermal lesions. Increased monocyte cytotoxicity was parallelled with an increased neutrophil cytotoxicity.

This study confirms earlier observations of increased chemotactic, phagocytic and bactericidal activity of monocytes and neutrophils in psoriatic patients (Krueger *et al.*, 1978b; Wahba *et al.*, 1978, 1979; Bar-Eli *et al.*, 1979; Sedgwick, Bergstresser & Hurd, 1980). Using a different target cell (leukemia EL$_4$ tumour cells) Bar-Eli *et al.* (1979) found normal antibody-dependent monocyte-mediated cytotoxicity in psoriatic patients. However, Bar-Eli *et al.* (1979) did not describe the severity or extent or psoriasis in their patients but, as shown in our study, increased cytotoxicity is correlated to the extent of epidermal lesions. Difference in selection of target cell type and effector to target cell ratio (Table 1) may also be of importance for the ADCC results.

Since cytotoxicity normalized after clinical remission the enhanced function of the phagocytic cells may be of a secondary nature. These results concur with the observations of Sedgwick *et al.* (1980) who showed that granulocyte adherence was positively correlated to the severity of psoriasis. Wahba *et al.* (1978, 1979) found increased neutrophil chemotaxis and phagocytosis in psoriatics irrespective of disease state and assumed that the altered neutrophil activity was of primary origin. However, investigations of neutrophil chemotaxis and phagocytosis in psoriasis have been controversial as increased (Wahba *et al.*, 1978, 1979), normal (Krueger *et al.*, 1978a) and even depressed function (Bork & Holzmann, 1974) have been reported. Leukocyte function might possibly be affected by the extracutaneous manifestations of psoriasis such as psoriatic arthritis and in this context Sedgwick *et al.* (1980) found that increased granulocyte adherence was more pronounced in psoriatic arthritis than in other psoriatics. However, in the present study monocyte cytotoxicity in arthritis patients did not differ from the other psoriatics.

Like monocyte cytotoxicity (Herlin & Kragballe, 1980), chemotactic and phagocytic functions are modulated by cyclic nucleotides *in vitro* (Cox & Karnovsky, 1973; Rivkin *et al.*, 1975). Thus increased cAMP/cGMP ratios will depress these functions and vice versa. It has been assumed that the increased chemotactic and phagocytic functions found in psoriatics are caused by a decreased cAMP/cGMP ratio (Wahba *et al.*, 1978, 1979; Bar-Eli *et al.*, 1979; Sedgwick *et al.*, 1980), but leukocyte cyclic nucleotide levels were not investigated. It was postulated that leukocyte cAMP/cGMP ratio was decreased as originally found in proliferating epidermal cells (Voorhees & Duell, 1971; Voorhees *et al.*, 1972, 1974). However, whether basal cAMP/cGMP ratio is decreased in psoriatic lesional epidermis is still controversial (Härkönen, Hopsu-Havu & Raij, 1974; Halprin *et al.*, 1975; Marcelo *et al.*, 1979; Adachi *et al.*, 1980) but it is generally accepted that cAMP levels are normal in uninvolved skin.

We found that in the same, highly purified cells assessed for monocyte and neutrophil cytotoxicity, the content of cyclic nucleotides was the same in the psoriatics as in normals. Recently, it has been stressed that monocytes from psoriatics have decreased cAMP content (Geerdink *et al.*, 1980) supporting the suggestions of Bar-Eli *et al.* (1979). However, Geerdink *et al.* (1980) investigated mononuclear leukocytes containing only 30% monocytes. The decreased cAMP level was therefore possibly confined to the contaminating lymphocytes as reported by others (Harpaz *et al.*, 1980).

A potent chemotactic activity for peripheral blood leukocytes has been shown in psoriatic scales (Tagami & Ofuji, 1976), possibly a C5 cleavage product. Also plasma from patients with widespread psoriasis markedly attracts normal human leukocytes (Wahba *et al.*, 1979). Therefore, an effect of patient serum on cytotoxicity mediated by normal monocytes is conceivable. However, preincubation of normal monocytes with fresh serum from psoriatics did not affect cytotoxicity.

Based on these considerations, we propose that the increased monocyte and neutrophil function in severe psoriasis is secondary to the disease activity. Furthermore, we have found that the increased monocyte and neutrophil cytotoxicity is not associated with altered basal cyclic nucleotide levels or influence on stimulatory serum factors. Although it is apparent that psoriasis is linked to altered monocyte and neutrophil function the precise mechanism of the interaction of phagocytes with proliferative epidermal cells has yet to be elucidated.

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Serum and urine concentrations of 5-methoxypsoralen after oral administration

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SUMMARY

Serum and urine concentrations were measured after oral administration of 5-methoxypsoralen (0·6 mg/kg or 1·2 mg/kg) to psoriasis patients. A dose of 0·6 mg/kg resulted in low serum concentrations, but after 1·2 mg/kg, much higher serum concentrations were reached. There was much individual variation in the time required for reaching peak serum levels. Minute amounts of unchanged 5-methoxypsoralen were excreted in urine. Much larger amounts were excreted as conjugates.

Orally administered 8-methoxypsoralen (8-MOP) (0·6 mg/kg body weight) and subsequent longwave ultraviolet irradiation has been used in recent years to treat severe psoriasis. Another psoralen derivative, 5-methoxypsoralen (5-MOP) is sometimes used instead of 8-MOP in photochemotherapy of psoriasis (Langner *et al.*, 1976; Hönigsmann *et al.*, 1979). Hönigsmann *et al.* (1979) reported 5-MOP, 0·6 mg/kg, to be as effective as 8-MOP. A higher dose of 5-MOP, 1·2 mg/kg, was even more effective. Only the high dose produced erythema. No nausea was experienced even after administration of the high dose of 5-MOP.

No information is available about 5-MOP in body fluids. In this study we determined the concentrations of 5-MOP in serum and urine after oral administration of micronized 5-MOP in gelatin capsules to psoriasis patients.

MATERIALS AND METHODS

Dosage forms

Crystalline 5-MOP* was micronized in the following way. One gram 5-MOP was dissolved in

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[*] 5-MOP (Bergapten) was kindly supplied by the Memphis Co., Cairo, Egypt. The drug was checked for purity by thin-layer chromatography and high performance liquid chromatography.

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about 40 ml chloroform and the resulting solution was mixed with 10 g microcrystalline cellulose (Avicel, PH 102†). The wet powder was stirred until the chloroform evaporated. The 5-MOP content of the powder was determined by high performance liquid chromatography. Microscopic examination showed a particle size of about 5 μm. The powder was accurately weighed and packed into hard gelatin capsules, so that each capsule contained about 10 mg 5-MOP. The capsules complied with the disintegration test of the Netherlands Pharmacopoeia, 8th edition.

Experimental procedure

Ten psoriasis patients participated in this study. No other medicines were taken 1 week prior to or during the tests. Just before administration a standardized breakfast was eaten, consisting of a glass of water and one jam sandwich. No additional food was allowed 10 h before and 4 h after administration. 5-MOP, 1·2 mg/kg, was administered to patients 1 to 7. Patients 8, 9 and 10 received a dose of 0·6 mg/kg. Blood samples were taken just before and at 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 4·0 and 5·0 h after administration. The serum fractions were separated and stored at −20°C until assayed. Urine was collected just before and in portions for 24 h after administration. The volume of the urine portions was measured and samples of about 10 ml were stored at −20°C until assayed.

Determination of 5-MOP in body fluids

5-MOP serum and urine concentrations were determined with high performance liquid chromatography, using a modification of a method for determination of 8-MOP (Stolk, 1980). Serum or urine (1 ml), after addition of 8-MOP as internal standard (100 ng), was extracted with heptane/dichloromethane 4:1. Chromatography was performed on a reversed phase C_{18}

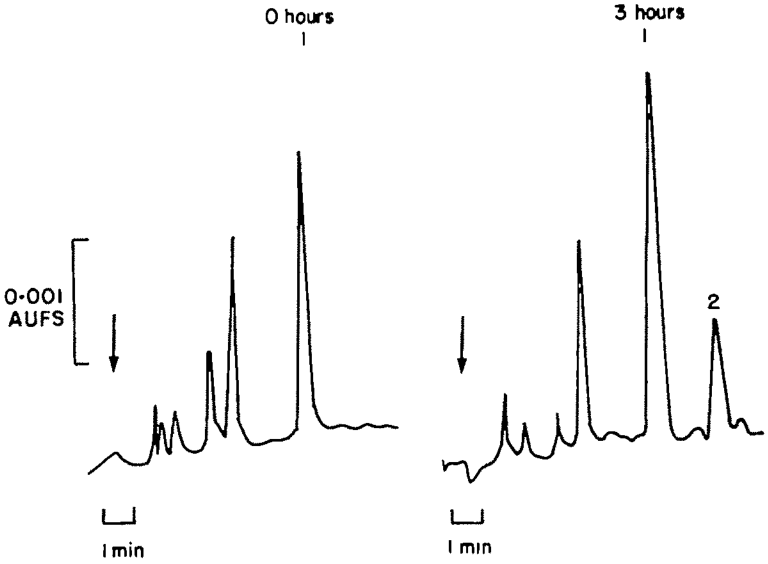

FIGURE 1. Chromatograms of serum samples of a patient taken before and 3 h after administration of 1·2 mg/kg 5-MOP: 1 = internal standard; 2 = 5-MOP (64 μg/l).

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† O.P.G., Utrecht, The Netherlands.

column with methanol/water 65 : 35 as mobile phase and UV-detection at 254 nm. Flow rate was 1 ml/min. Determination of the serum levels was based on peak height measurements.

A standard curve was prepared by adding 50, 100, 150 and 200 ng 5-MOP to 1 ml control serum, each containing the same amount of internal standard. 5-MOP was determined at each concentration four times. A good linear correlation was observed between the peak height ratios of 5-MOP/8-MOP and the 5-MOP serum concentrations: 0·9999 ($n = 16$).

The coefficients of variation determined at 50, 100, 150 and 200 ng 5-MOP were respectively 6, 2, 7 and 4%. The detection limit of the assay was about 5 ng/ml serum. Chromatograms of serum samples of one patient, before and 3 h after intake of 1·2 mg/kg 5-MOP, are shown in Fig. 1.

Hydrolysis of urine samples

To 1 ml urine in a 10 ml test tube, 1 ml hydrochloric acid 36% was added, and the mixture was heated on a boiling water bath for 15 min. The mixture was diluted with distilled water to 100 ml. Then 1 ml of the dilution was determined by HPLC. Hydrolysed urine samples were also analysed by thin-layer chromatography. In that case the mixture after hydrolysis was extracted with 5 ml heptane/dichloromethane 4 : 1. After evaporation the extract was applied to a thin-layer plate (kieselgel G) and developed in hexane/ethylacetate 7 : 3. The spots were detected by fluorescence under short wave UV-light.

Pharmacokinetic parameters

The area under the serum concentration curves (AUC) from 0 to 5 h was calculated by the trapezoid rule.

TABLE 1. Maximum serum concentration (C_{max}), time for serum peak concentration (T_{max}), area under the curve (AUC) and urine excretion after administration of 0·6 mg/kg (patients 8–10) and 1·2 mg/kg (patients 1–7) 5-MOP

Patient	Sex	Weight (kg)	Age (years)	C_{max} (μg/l)	T_{max} (h)	AUC_{0-5} (μg/h/l)	0–24 h urine excretion (μg)
1	M	87	61	400	4	736	n.d.
2	M	93	52	257	4	412	4·7
3	M	76	30	380	4	613	8·6
4	M	68	52	500	2	1511	7·4
5	M	65	30	530	3	1025	6·0
6	M	71	24	475	3	1540	n.d.
7	F	61	47	298	2·5	587	2·0
Mean		74	42	406	3·2	918	5·7
±				103	0·8	455	2·5
8	M	84	69	64	2·5	88	n.d.
9	M	92	40	152	3	284	n.d.
10	M	65	49	61	3	97	n.d.
Mean		80	53	92	2·8	156	
±				52	0·3	111	

n.d. = Not determined.

RESULTS AND DISCUSSION

Table 1 demonstrates the values of the serum peak concentrations, C_{max}, the AUC, the time required to reach C_{max}, T_{max} and the total amount of unchanged 5-MOP excreted in urine during 24 h. Only low serum concentrations were measured after administration of 0·6 mg/kg to patients 8, 9 and 10. However, after administration of 1·2 mg/kg (patients 1–7) much higher serum concentrations were measured. Hönigsmann *et al.* (1979) observed erythema only after administration of the 'high' dose, 1·2 mg/kg, and subsequent UV-A dosimetry. This suggests a correlation between 5-MOP serum level and erythema. Serum concentration curves of patients 1–7 are shown in Fig. 2. The serum concentration curves indicate both a rapid absorption and elimination. From some curves the half life for elimination could be measured. The half life of the 5-MOP serum concentrations of patients 2, 5 and 7 was respectively 105, 73 and 45 min. Probably the real half life is shorter, because the absorption seems to go on after serum peak concentration. This makes 5-MOP a high clearance drug, like 8-MOP.

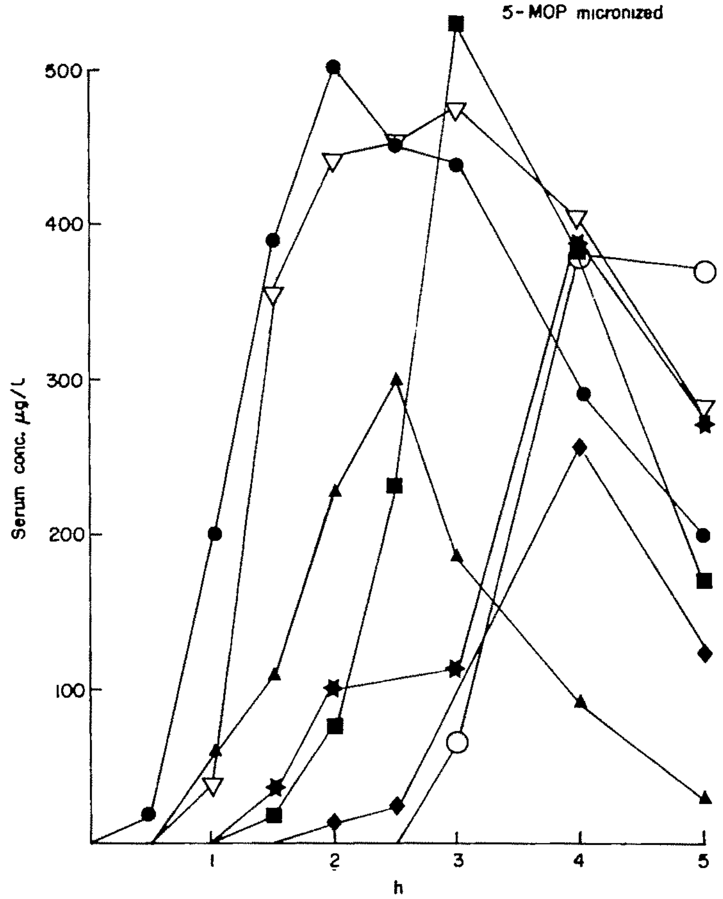

FIGURE 2. Serum concentration curves for 5-MOP after oral administration of 1·2 mg/kg. Patient no: 1—✱; 2—◆; 3—○; 4—●; 5—■; 6—▽; 7—▲.

The mean time for reaching serum peak levels is 3·2 h (1·2 mg/kg). However, there is very

TABLE 2. Excretion of 5-MOP conjugates

Patient 2		Patient 3	
Hours after administration	5-MOP excreted (μg)	Hours after administration	5-MOP excreted (μg)
0–2·5	886	0–2	170
2·5–8	1928	2·5–6·45	381
8–14	990	6·45–12	1102
14–21	612	12–14	345
		14–22	738
Total	4416	Total	2736

much individual variation of T_{max}. The reason is probably the very poor water solubility of 5-MOP. Other methods of administration with more predictable absorption characteristics are now being investigated. Very small amounts of unchanged 5-MOP are excreted in urine. After acid hydrolysis of urine samples a much larger amount of 5-MOP could be detected with HPLC analysis. The presence of 5-MOP was confirmed with thin-layer chromatography. The amount excreted in urine portions during about 24 h by patients 2 and 3 was measured (Table 2). Probably 5-MOP is here excreted as conjugates (e.g. glucuronate and sulphate) just as Ehrsson, Eksberg & Wallin (1978) observed for 8-MOP. The detailed structure of these conjugates has not been established, but it is reasonable to assume that conjugation occurs at the carboxylic or phenolic groups of hydrolysed 5-MOP (5-MOP-acid). 5-MOP-acid liberated during hydrolysis will lactonize to 5-MOP and unchanged 5-MOP can be determined. Urine excretion of the conjugates goes on long after disappearance of 5-MOP from the serum. The conjugates were already detectable in urine collected 0–2 h after administration to patient 3.

Measurable serum concentrations of 5-MOP were observed only 2·5 h after administration. This indicates rapid biotransformation just after absorption, possibly in the wall of the intestine or the liver. In this way 5-MOP may be metabolized before reaching the general circulation.

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Serum 25-OH vitamin D after photochemotherapy

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SUMMARY

Patients receiving photochemotherapy (PUVA) for psoriasis were shown to have an increase in serum 25-OH vitamin D. The effect was not seen with UV-A alone or 8-MOP alone.

In a preliminary study (Rogers, Marks & Shuster, 1979) we found an increase in the blood concentration of 25-hydroxy vitamin D (25-OHD) in patients with psoraisis treated by photochemotherapy with 8-methoxypsoralen (8-MOP) and long-wave UV irradiation (UV-A, 320–400 peak wavelength 365 nm). This observation was surprising because it is believed that vitamin D_3 synthesis from 7-dehydrocholesterol is promoted in the epidermis by UV-B (280–310 nm). The present study was therefore done to confirm the original observations and to see whether the effect was due to the augmentation of UV-A action by 8-MOP or by UV-B produced by the UV-A irradiation unit.

PATIENTS, METHODS AND RESULTS

Eight men and five women aged 19–65 years (mean 41·2 years ± 16·4 s.d.) with discoid psoriasis were studied before and during treatment with 8-MOP and UV-A (PUVA). The 8-MOP was given orally on a body weight basis and UV-A as predicted from skin type as previously described (Rogers *et al.*, 1979). Blood was taken for 25-OHD estimations at approximately the same time each morning before treatment and 24 h after UV-A alone without previous 8-MOP and 24 h after administration of 8-MOP alone without UV-A and after administration of both (26 h after 8-MOP and 24 h after UV-A) (8-MOP was given alone after UV-A alone to exclude any effect due to its persistance). UV-A irradiation was from the same Waldmann 4000 or 6000 units used in previous studies (Rogers *et al.*, 1979). Serum 25-OHD was measured by competitive protein binding (Greenberg *et al.*, 1974).

The results (Fig. 1) show a slight increase in serum 25-OHD after UV-A alone and a slight increase after 8-MOP alone which was not statistically significant, and a large and significant increase after 8-MOP and UV-A in combination. A slightly smaller increase was also found on two subsequent occasions after PUVA. The magnitude of the increase was not related to skin type but the range of skin types in the study was too small to exclude a relationship.

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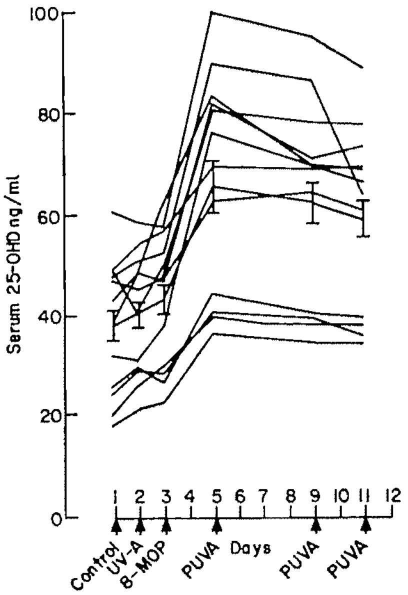

FIGURE 1. Serum 25-OHD (ng/ml) levels in thirteen patients before and after PUVA. Vertical lines and bars show mean ± s.e.mean for each group (see text).

DISCUSSION

The results confirm our previous observation of an increase in serum 25-OHD after photothemotherapy with 8-MOP and UV-A. The absence of any effect after UV-A alone and 8-MOP alone shows that the effect of PUVA on epidermal vitamin D_3 synthesis is due to the combined action of UV-A and 8-MOP. This also excludes the possibility that the effect of PUVA was due to a direct action (independent of 8-MOP) of adventitious UV-B emitted by the irradiation unit. These findings show that contrary to current beliefs either the action spectrum of 8-MOP is not limited to UV-A, or that the synthesis of vitamin D_3 in the skin is promoted by UV-A as well as UV-B. Resolution of this problem will require studies of the action spectrum of 8-MOP and of the synthesis of vitamin D_3 in the epidermis. This may have important theoretical consequences and may also help to optimise photochemotherapy. As in our previous study the increase in serum 25-OHD was not of the order known to produce toxic effects but there may be situations in which the response is therapeutically useful.

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The spectrum of inflammatory skin disease following jejuno-ileal bypass for morbid obesity

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SUMMARY

From a series of 105 patients who had undergone jejuno-ileal bypass for morbid obesity, seven developed an episodic illness featuring inflammatory skin lesions, usually associated with a non-destructive polyarthritis. Tenosynovitis, myalgia and fever had also occurred. The illness abated spontaneously in four patients.

The most characteristic skin lesion was an erythematous macule which developed a central vesicle or pustule. Erythema nodosum-like lesions and liquefying nodules were also seen. Early lesions were characterised by a dense accumulation of neutrophils. Changes in dermal vessels were less destructive than is usually seen in immune complex mediated vasculitis. Circulating immune complexes could be demonstrated in patients actively developing skin lesions. They were also found in some patients long after activity had ceased and in jejuno-ileal bypass subjects who had never developed inflammatory lesions.

Dapsone was effective in suppressing the skin and joint symptoms when metronidazole and/or tetracycline had failed.

The use of jejuno-ileal (JI) bypass procedures for morbid obesity has been accompanied by a wide range of adverse reactions. The recognised cutaneous complications include vitamin A deficiency, probably due to rapid transit time and reduced absorptive surface (Wechsler, 1979), dependent oedema from hypoproteinaemia, dry scaly skin which may be partially related to essential fatty acid deficiency, and hair loss, usually temporary and most probably telogen effluvium. Inflammatory skin lesions have been referred to in several series describing medium and long term sequelae of JI bypass but only recently have there been detailed descriptions of the clinical and pathological features. From these more recent reports it is clear that there are a variety of different types of skin lesion, and a common association with arthralgia, arthritis, tenosynovitis, myalgia, fever and sometimes other systemic manifestations. Although it has been proposed that these inflammatory reactions are a consequence of circulating immune complexes which contain bacterial antigen (Wands *et al.*, 1976) there is no conclusive evidence for such a mechanism in most cases.

In the study reported here, the aims were to examine all patients in one centre who developed skin disease after JI bypass. This would allow an estimate to be made of the prevalence of inflammatory

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skin lesions and associated phenomena, their nature, and therapeutic measures which influence the natural history. The results of immunological and other investigations during phases of activity and quiescence in individual patients have been compared. The same tests have been used in a group of JI bypass patients who have been free of lesions thus far.

MATERIALS AND METHODS

Patients

All patients who have had JI bypass for morbid obesity at St George's Hospital were sent a questionnaire and stamped addressed envelope for their reply. Those who replied that they had had skin trouble since JI bypass were summoned for clinical review unless their reply indicated that their skin disease could not have been bypass-related, e.g. basal cell carcinoma.

Investigative procedures

Full blood count, liver function tests, immunoglobulins, CH_{50}, C_3 and C_4 were evaluated by routine laboratory procedures. Cryoprecipitates were sought by collecting blood into bottles warmed to 37°C, centrifugation at 37°C after the blood had clotted, and storage of sera at 4°C for up to 7 days. Cryoprecipitates were centrifuged, resuspended and kept at 37°C overnight, and stored at 4°C for reprecipitation over a further 3 days.

Immune complexes were obtained by 2% polyethylene glycol precipitation from serum and analysed for immunoglobulin and complement content (Dambuyant, Burton-Kee & Mowbray, 1978).

From each skin lesion biopsied a portion was sent for aerobic and anaerobic bacterial culture; the rest was formalin fixed and paraffin embedded. Sections were stained by haematoxylin and eosin and by methods to demonstrate connective tissue elements, fibrin and bacteria.

In addition some biopsied lesions that were less than 24 h old were snap frozen in OCT medium in solid CO_2-chilled *n*-hexane and stored at −70°C for subsequent direct immunofluorescence. This was performed using standard techniques and adequate controls (Beutner *et al.*, 1973).

Intradermal histamine injection can be used to localize circulating immune complexes (Braverman & Yen, 1975). In selected patients, from whom informed consent was obtained, 0·05 ml 1:1000 histamine sulphate was injected intradermally into the flexor surface of the forearm and 4 mm punch biopsies taken approximately 2 hours later. At control sites 0·05 ml physiological saline was injected and biopsies obtained. The punch biopsies were snap frozen in OCT medium in chilled *n*-hexane and stored at −70°C for subsequent direct immunofluorescence and histology.

RESULTS

Survey data

Of the 105 patients who were sent the questionnaire, eighty-five replied. Forty-two denied any change in their skin since JI bypass and nineteen gave either a definite diagnosis or a description which excluded the possibility of inflammatory skin disease. This group included four complaining of dry skin and one with marked hair fall. Of the remaining patients, thirteen came for clinical assessment. From the history and examination a diagnosis of JI bypass inflammatory skin disease was made in seven cases. In five there was evidence of arthralgia or arthritis.

A comparison between the seven patients and the rest of the St George's series with respect to age, sex ratio, post-operative weight loss and other JI bypass complications (e.g. liver disease) revealed no significant differences. All patients had end-to-side JI anastomosis.

None of the cases had any past or family history of either skin or joint disease.

Natural history of inflammatory skin lesions

There was an interval of 6 weeks to 3 years between JI bypass and the onset of symptoms. In all but two cases, the skin lesions (and other symptoms when present) ceased after periods ranging from 7 months to 4 years. This apparently permanent cessation of symptoms was unrelated to therapy (see below). Numbers of lesions per episode ranged from less than 5 to more than 50. The frequency of cropping varied from more than one per week to intervals of 6–9 months between crops (patient J.E.). In one case (V.R.) crops occurred regularly one week before menstrual periods. In another case (J.L.) there was a conviction that certain foods precipitated lesions, but subsequent challenge failed to elicit lesions. Otherwise there was no discernible pattern to the appearance of new lesions. In one case (D.W.) the severity of symptoms led to reversal of the bypass and prompt resolution of skin and joint disease.

Skin lesions

Lesions have been seen at most sites except the palms, soles, face and genitalia. None have been seen on mucous membranes. The most common sites were the extensor surface of the arms, the thighs, buttocks and lower abdomen.

Some patients were aware of discomfort or even pain in the skin up to a few hours before lesions were visible. The earliest lesion was usually an erythematous macule, up to 1 cm across. Some macules resolved without trace in less than 24 h while others developed a 1–2 mm diameter central vesicle or pustule (Fig. 1). Such lesions lasted a few days and usually healed without scarring. Lesions with larger pustules healed leaving shallow scars.

Lesions resembling erythema nodosum have also been seen. These were oedematous, erythematous, readily palpable and often tender (Fig. 2). Sometimes these also developed central pustules and often healed with scarring. Nodules usually persisted at least 1–2 weeks.

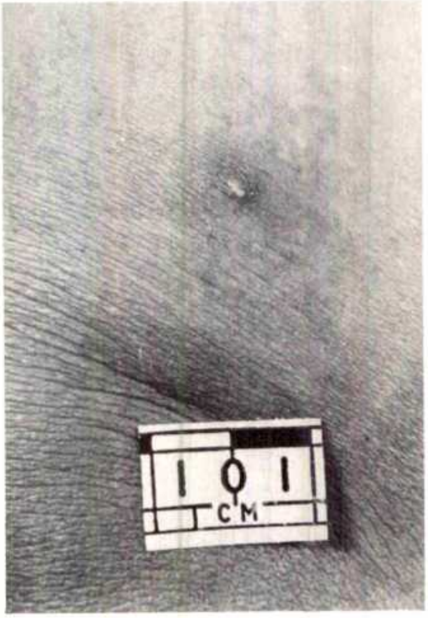

FIGURE 1. Pustule with erythematous halo.

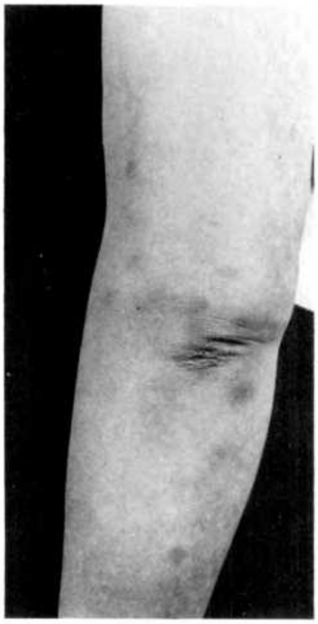

FIGURE 2. Palpable erythematous lesions on the arm.

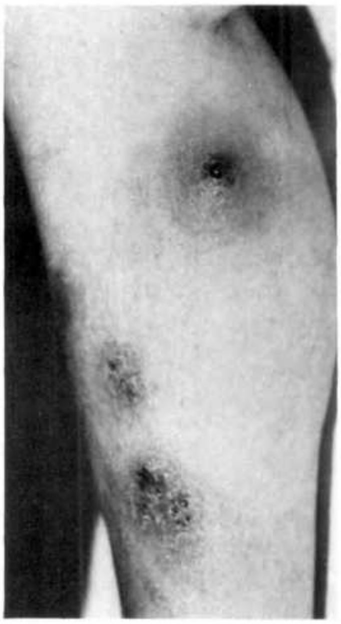

FIGURE 3. Liquefying panniculitis on the lower leg.

In one case (J.L.) there were deep tender nodules with little or no surface change until necrotic material was discharged through them (Fig. 3). These took several weeks to heal, leaving marked scarring.

Joint symptoms

Joint pains usually accompanied crops of skin lesions but sometimes occurred independently. Typically, one or a few joints became very painful with very little or no decrease in the range of passive movement but often with capsular tenderness. Swelling and effusions were seen frequently in two cases, one of whom (D.W.) required reanastomosis because of the severity of symptoms. The joints most commonly affected have been knees, wrists, fingers, elbows and ankles. There have been no significant radiological changes other than soft tissue swelling. As with the skin lesions, joint symptoms have been episodic and self limiting.

Other episodic symptoms

Three cases (J.E., P.B. and D.W.) have had fever with most of their episodes of skin lesions. This was usually associated with malaise and myalgia. From the histories, two cases (D.W. and P.B.) have probably had tenosynovitis of the hands and wrists.

Effect of antibiotics

All patients have been given metronidazole (600 mg daily) and tetracycline (1 g daily) separately or combined for periods of a few days to several weeks. One patient (J.E.) had marked benefit from the prompt use of tetracycline but did not find that prolonged treatment prevented episodes. Metronidazole produced doubtful prophylaxis in two patients. In the remaining cases there was no obvious reduction in frequency or severity of episodes.

The two patients with greatest frequency of episodes (P.B. and L.S.) have also been treated with dapsone (diamino diphenyl sulphone). The initial daily dose was 150 mg and the maintenance dose 50–100 mg. In both patients this drug aborted episodes in 12–24 h and during 3 periods of 4–6 weeks on dapsone, symptoms were either abolished or reduced. Episodes recurred within 1 week of discontinuing dapsone on each occasion. No adverse effects were noted.

Routine haematological and biochemical tests

During episodes of skin and or joint symptoms the sedimentation rate was often elevated to 30–50 mm/1 h. No other consistent abnormalities were noted.

Immunological abnormalities

Three patients have been evaluated while actively producing lesions and while quiescent (Table 1). The other four patients have been evaluated only while quiescent (Table 2). Five patients whose JI bypass has not been complicated by skin or joint disease have also been evaluated (Table 3). Table 1 indicates that circulating immune complexes were present while new lesions were appearing; with patient P.B. total haemolytic complement during episodes was depressed and rose to within normal limits or above during quiescence. Immune complexes were also found, however, between episodes, in patients no longer developing lesions and in the control subjects. C_3 and C_3 showed no significant deviation from normal.

TABLE 1. Immune complex evaluation: patients with skin lesions

		Immune complex constituents	
Patient	Date	Active	Inactive
L.S.	11/8/78	$C1_q$, IgG, IgA	
	15/4/79	IgG, IgA	
	13/6/79		None
	27/7/79		None
	21/8/79		None
P.B.	8/2/79		IgA
	16/2/79	IgG, IgA	
	26/2/79	IgG	
	13/6/79		None
	23/7/79		None
J.L.	24/5/77	$C1_q$, IgG	
	2/3/79		$C1_q$ IgG, IgM

TABLE 2. Immune complex evaluation: past history of skin lesions

Patient	Immune complex constituents
J.E.	None
D.W.	IgA
L.A.	None
V.R.	None

TABLE 3. Immune complex evaluation: controls

Patient	Date of bypass	Immune complex constituents
1	1973	None
2	1973	$C1_q$, IgG, IgA
3	1974	IgA
4	1976	None
5	1976	IgG, IgA

In no cases were cryoprecipitates found in other than very small quantities and they were not analysed further.

Direct immunofluorescence

In the two cases (P.B. and L.S.) examined while lesions were appearing, IgM and C_3 were demonstrated in blood vessel walls. Identical findings were produced in normal skin injected with histamine. It should be noted that a further histamine injected site in each patient was observed over a 36-h period. Following the immediate weal and flare reaction, there were no visible or palpable abnormalities.

Histopathology

Nine biopsies from six patients have been examined. The early macular lesion showed dilated superficial dermal venules and capillaries surrounded by neutrophil polymorphs (Fig. 4). Early vesicular and pustular lesions showed similar vascular changes, a greater accumulation of polymorphs and

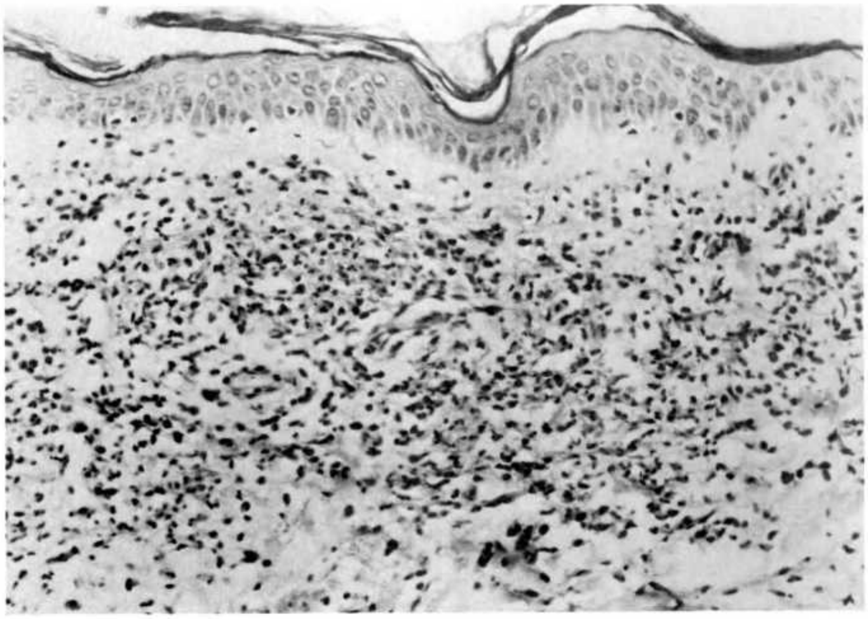

FIGURE 4. Predominantly neutrophil polymorph infiltrate in the superficial dermis (H & E, × 952).

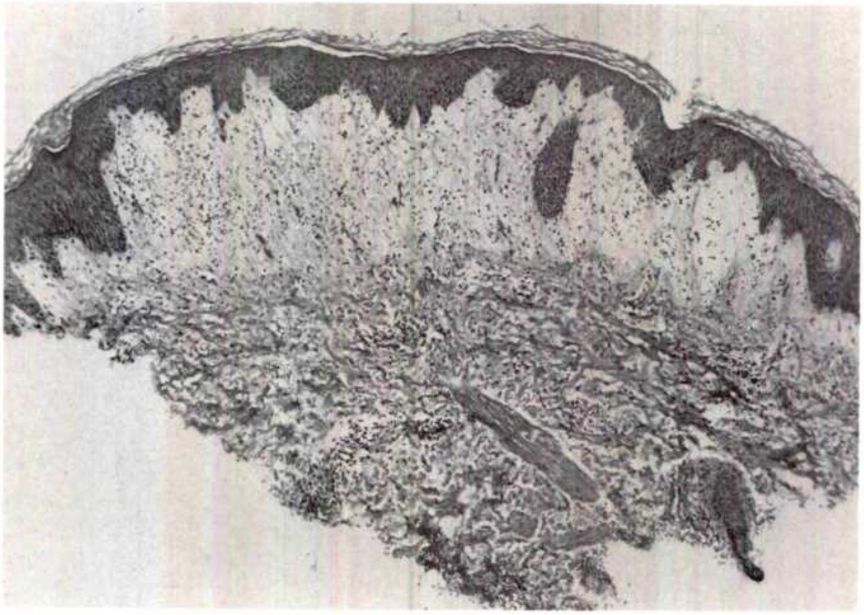

FIGURE 5. Oedema of the superficial dermis leading to dermo-epidermal separation (H & E, × 158).

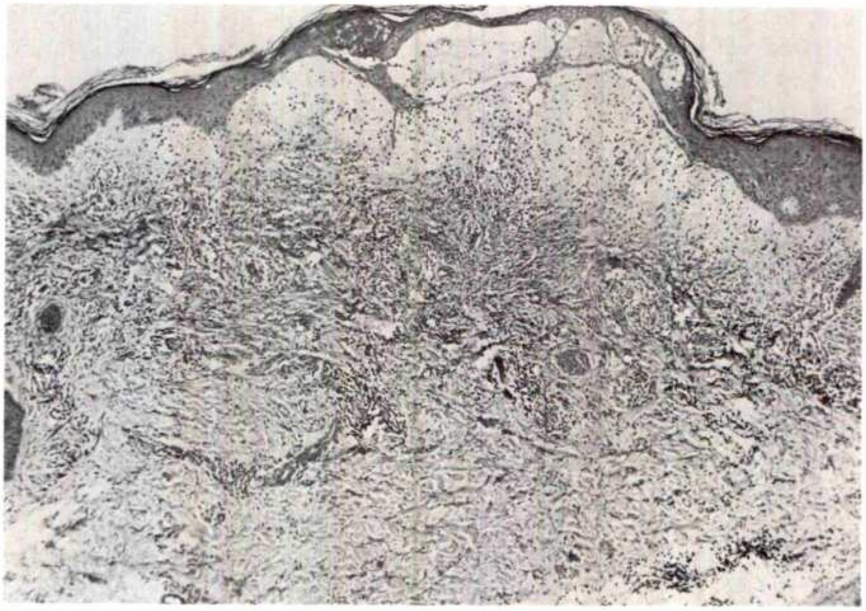

FIGURE 6. Necrotic changes in the epidermis with oedema and a neutrophil polymorph exudate beneath (H & E, × 154).

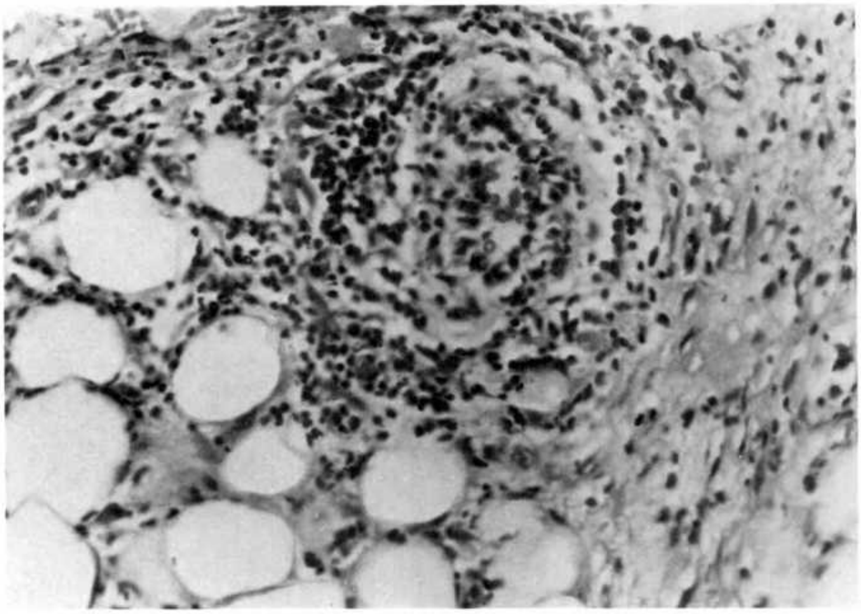

FIGURE 7. Neutrophil polymorphs and mononuclear cells around a septal venule (H & E, × 1400).

intense oedema of the papillary dermis leading to dermo-epidermal separation (Fig. 5). In some biopsies the epidermis showed necrotic changes without vascular occlusion or epidermal separation (Fig. 6). One example of the larger pustular lesions showed the histological changes of an acute purulent folliculitis. In lesions with dense accumulations of polymorphs there was swelling of endothelial cells in the adjacent venules, but in no case was fibrinoid necrosis of vessel walls observed. Early lesions resembling erythema nodosum showed perivascular neutrophil polymorphs in the fat septa and deep dermis; at a later stage a predominantly mononuclear cell infiltrate was seen (Fig. 7). The biopsies from the case with liquefying nodules showed the changes of fat necrosis, chronic inflammation and fibrosis but no vasculitis. Early lesions from this case were not examined.

All material sent for bacterial culture was sterile.

DISCUSSION

Approximately 7% of those who have had JI bypass at St George's Hospital have developed a variety of different morphological types of inflammatory skin lesions, usually associated with joint pains. The most common histopathological event in the skin is an influx of neutrophil polymorphs leading to sterile pustule formation. Although the dermal capillaries and venules show mild degrees of damage, necrotising vasculitis has not been a feature. The condition has been episodic and in most cases self-limiting.

Many thousands of small bowel bypass procedures have been performed in the U.S.A. alone (Iber & Copper, 1977) and there have been hundreds of papers devoted to the techniques and complications. Although rheumatic complications have been noted in many series, often with a prevalence of about 20%, there have only been sporadic accounts of inflammatory skin lesions (Wills, 1972; Drenick *et al.*, 1976; Wands *et al.*, 1976; Campbell *et al.*, 1977; Ely, 1978; Dickson, Aoyama & Wilkens, 1978; Hardy, 1978; Utsinger *et al.*, 1978; Goldman *et al.*, 1979; Dicken & Seehafer, 1979; Morrison & Fourie, 1980). The most commonly described skin lesion has been a vesicle or pustule on an erythematous base although nodules resembling erythema nodosum and erythematous macules have also been reported. Most of these cases have also had the associated episodic rheumatic and febrile symptoms described in the present series.

We have found a range of pathological changes in the skin. It is likely that in part these reflect differences in the anatomical site at which the inflammatory process is initiated, and also the variable time elapsed between onset and skin biopsy (Ryan, 1976). The common denominator in early lesions is an accumulation of neutrophil polymorphs. There is mild or minimal change in vessel walls in contrast to the destructive changes seen in many well defined instances of immune complex vasculitis, although leucocytoclastic vasculitis has been reported (Dickson *et al.*, 1978; Goldman *et al.*, 1979).

Either as an antigen in immune complexes or by some other mechanism, it is likely that bacterial products play an important role in the pathogenesis of the inflammatory complications of JI bypass. Treatment with antibiotics is often successful in treatment and prevention, and reversal of the operation brings about a prompt resolution of complications, as was seen in D.W. The normal jejunum is either sterile or has small amounts of aerobic bacteria. After bypass the excluded loop and the functioning bowel become colonized by large quantities of faecal flora including anaerobes such as *Bacteroides* species (Corrodi *et al.*, 1978). In addition to the arthritis and skin lesions, this change in flora has been implicated in the pathogenesis of the enteropathy (Drenick *et al.*, 1976) and hepatic disease (Maxwell, 1980).

In three patients with bypass-associated arthritis, one of whom had an 'erythematous macular rash', Wands *et al.* (1976) found circulating cryoproteins only during phases of disease activity. The cryoproteins were shown to be immune complexes containing antibodies reactive with *Escherichia coli* and *Bacillus fragilis*. During episodes of arthritis there was also evidence suggesting activation of the alternative pathway of complement*. Several workers have used direct immunofluorescence techniques to implicate the circulating immune complexes in the genesis of skin lesions. IgG and C3 have been demonstrated in vessel walls (Utsinger *et al.*, 1978) and in the basement membrane region (Dickson *et al.*, 1978; Hardy, 1978; Dicken & Seehafer, 1979), but several authors have reported negative findings. In our series two cases had IgM and C3 in vessel walls of early lesions, and yet at the time the biopsies were taken the circulating immune complex material did not contain IgM. The results are difficult to interpret. In some cases negative results may simply be a consequence of biopsying lesions too late (Cream, 1971), and false positive results can occur following vasodilatation or vascular damage allowing circulating macromolecules to leak into the extravascular space (Ryan, 1976). Neither in our series, nor to our knowledge elsewhere, has a putative antigen been demonstrated at the site of injury in the skin.

The skin and musculoskeletal tissues are the most common target structures for JI bypass related inflammatory disease, but renal damage (Drenick *et al.*, 1978) and intravascular haemolysis, thrombocytopenia and leucopenia (Moake *et al.*, 1978) have been reported in association with circulating immune complexes.

Even when circulating immune complexes are not demonstrable, there may be evidence of activation of the alternative pathway of complement (Moller, Jensen & Nielson, 1979). Bacterial lipopolysaccaride could be responsible for this, without the mediation of antibody, and similarly could account for the neutrophilic inflammation in the skin lesions (Abdulla & Schwab, 1966; Hadler & Granovetter, 1978).

Why do only some patients develop inflammatory skin and joint disease after JI bypass, and why is the condition usually self-limiting? At present little is known to help answer these questions. It has been suggested that the surgical technique may be important, end-to-end anastomosis being more likely to be associated with stagnation and bacterial proliferation in the excluded loop than the end-to-side method as used in our series (Goldman *et al.*, 1979). This explanation seems unlikely; there is no excess of arthritis or skin disease in a recent large end-to-end series (Halverson *et al.*, 1978) compared with several end-to-side series. Other possibilities which we hope to explore include the definition of a susceptible HLA haplotype and paralysis of the mononuclear phagocyte system, particularly in the liver, allowing bacterial debris and/or immune complexes access to the general circulation.

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* The presence of IgA in immune complexes during periods of disease activity in some of our cases is suggestive of a gastrointestinal origin for the antibody component.

Is there a naturally occurring counterpart to the inflammatory disease seen after JI bypass? The clinical and histological features of JI bypass skin lesions have been closely mimicked in ulcerative colitis (Johnson & Wilson, 1969; Basler & Dubin, 1976; O'Loughlin & Perry, 1978). Erythema nodosum, in its most characteristic location on the shins, is usually associated with exacerbation of the colitis and joint symptoms. Erythematous macules and papules progressing to pustules have been described as the sole cutaneous manifestation of ulcerative colitis and also as a precursor of pyoderma gangrenosum (Basler & Dubin, 1976). Pustular eruptions are usually associated with very active ulcerative colitis, fever and arthralgia. Circulating immune complexes are present in greater quantities at such times but their pathogenetic role remains to be established (Hodgson, Potter & Jewell, 1977). Clinical differences between the pustular lesions of JI bypass and ulcerative colitis include a tendency for the latter to occur on the face and in the mouth and bypass associated pustules do not progress to pyoderma gangrenosum. Histology of skin lesions is very similar in the two conditions, notably the intense neutrophil accumulations, sometimes follicular or perifollicular, and the rarity of necrotizing vasculitis.

The JI bypass skin lesions and joint symptoms usually respond to treatment with tetracycline and/or metronidazole, although several authors have noted a lack of response, and then used corticosteroids. In two patients in whom antibiotics were unsuccessful dapsone proved to be beneficial and well tolerated. Morrison & Fourie (1980) have also noted the value of dapsone. Since there is usually a self-limiting natural history, it would seem prudent to use anti-inflammatory drugs rather than reverse the bypass in patients who have otherwise benefitted from substantial weight loss.

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Nickel, hands and hip prostheses

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SUMMARY

The evidence on the effect of oral nickel on hand dermatitis in nickel sensitive patients is conflicting. The evidence of this study suggests that the amount in an ordinary diet plays no part in the chronicity of nickel dermatitis although this conclusion must be made with reservations because of the small number of patients in the present study. It may be that larger doses not found in a normal diet may have some aggravating effect. Low nickel diets are difficult due to the widespread distribution of nickel in food. There is no evidence that current metals on plastic hips sensitize to nickel or aggravate existing dermatitis. Nickel allergy is not a factor in rejection so patients who are allergic to metal do not necessarily require titanium hips and pre-operative patch testing is unnecessary.

The administration of nickel, either orally or by implantation, is of importance to dermatologists because:

(1) Nickel ingested may have an aggravating effect on nickel dermatitis.

(2) Joint implants may sensitize to metals or aggravate existing dermatitis, or more important, metal sensitization may lead to rejection of joint implants.

(3) About 10% of women are allergic to nickel (Kieffer, 1979).

ORAL NICKEL

There are a number of factors which suggest that nickel systemically may aggravate existing dermatitis:

(1) Nickel dermatitis often occurs on palms, particularly the distal palms and not the more usual backs and sides of the fingers. However, as most contact with nickel is with solid objects this might be expected.

(2) Nickel dermatitis of the hand can be unusually chronic, pointing to a repeated stimulus. Nickel is so widely present in metal that external contact would be expected to be frequent.

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(3) Anecdotal—aggravation of nickel dermatitis has been reported on swallowing a coin. The dermatitis improved on removing the coin (Lacroix, Morin & Collin, 1979).

(4) Aggravation of nickel dermatitis with metal implants (Oleffe & Wilmet, 1980).

Several investigative studies have been carried out in an effort to resolve this problem:

(1) Urinary levels of nickel have been found increased during exacerbations (Menne & Thorboe, 1976). These authors studied four patients only, too few to have statistical significance and there were not adequate controls.

(2) Kaaber, Veien & Tjell (1978) investigated the effect of a low nickel diet on seventeen patients with nickel dermatitis. Three cleared, six improved and eight showed no improvement. There are two major difficulties which apply to this type of study: (a) nickel is fairly universally dispersed throughout food and it is difficult to reduce the content of nickel by more than fifty per cent (Schroeder, Balassa & Tipton, 1962); (b) the course of nickel dermatitis is so variable that assessment of this type of study is difficult.

(3) Nickel chelation: Kaaber *et al.* (1979) treated eleven patients with Antabuse which is an effective nickel chelating agent. Seven of them cleared, two improved and two remained unchanged. The numbers were small and there were no controls.

(4) An increase in nickel sensitization after metal implants (Benson, Goodwin & Brostoff, 1975; Elves *et al.*, 1975; Munro-Ashman & Miller, 1976).

(5) The only satisfactory investigation is a double-blind trial of oral nickel in patients with nickel hand eczema. There have been three studies. Christensen & Moller (1975) gave 5·6 mg nickel to twelve patients alternating this with placebo in a double-blind trial. With the placebo there was no flare-up; with nickel, nine were worse and three showed no change. Kaaber *et al.* (1978) gave twenty-eight patients placebo and then 3 days later gave 2·5 mg of nickel. The condition of those on placebo did not worsen but of those on nickel, seventeen were worse and eleven showed no change. The treatments were not randomly allocated, all patients receiving placebo first. Jordan & King (1979) gave ten patients 0·5 mg of nickel daily for 2 days for 1 week and lactose the second week, or vice versa depending on the random allocation, assessing the number of flares. This regime was repeated in reverse order for 2 further weeks. They found seven had flares with twenty 2-day nickel supplements, and five had flares with twenty 2-day placebo supplements. There was, then, no significant connection between oral nickel and dermatitis flares.

There is, therefore, one double-blind random study (Christensen & Moller, 1975) suggesting a direct connection between oral nickel and aggravation of hand dermatitis, and one suggesting no connection (Jordan & King, 1979) (a total of twenty-two patients). There are some important differences in these studies:

(1) Christensen & Moller (1975) gave a much larger dose of nickel than Jordan & King (1979). The nickel contents of diets vary but the evidence would tend to suggest that an average daily diet contains 0·5–0·75 mg nickel (Shroeder *et al.*, 1962; Committee on Medical and Biological Effects of Environmental Pollution, 1975); therefore Jordan & King's (1979) dose would correspond with the type of intake expected in a person with nickel dermatitis.

(2) Christensen & Moller (1975) assessed their patients by counting the number of blisters before and after testing; Jordan & King (1979), assessed patients' opinion and general clinical opinion. Counting the blisters may seem a more accurate way of assessment but a small study carried out in our Department showed a wide variation in different observers and in the same observer from time to time. So, the patients' and doctors' opinion of flares may in fact be more accurate, particularly in a double-blind trial.

INVESTIGATION

Twenty-two patients with palmar dermatitis and a positive nickel patch test were entered in a 4-week trial. They were given two capsules twice daily for 2 consecutive days on Tuesday and Wednesday. These capsules contained either lactose or nickel sulphate, containing 500 μg of elemental nickel. The patients were randomly allocated to either nickel or lactose first. They were assessed for worsening of their hands on Wednesday and Friday. The second week they received the reverse capsules, and the third and fourth week the same capsules again in the same order but in double the dose. That is, in the first fortnight they received 2 mg of nickel sulphate on two days, i.e. a total of 4 mg, and on the second trial period 4 mg daily, that is, in 2 days 8 mg. The study was performed double-blind. Urinary nickel was estimated as follows: the sample was subjected to acid digestion (Nomoto & Sunderman, 1970) and nickel extracted went into methyisobutylketone as pyrrolidine-dithiocarbamate complex. Nickel was measured by atomic absorption using a Perkin and Elmer model 370 Spectrophotometer equipped with a graphite furnace. Despite repeated efforts nickel could not be detected in the serum using the same or other methods.

RESULTS

Urine samples were taken at each visit and analysed for nickel. The urinary nickel levels were found to increase following ingestion of nickel—the highest level being 66 μg/l with mean levels of 13·8 (s.d. 13·6) and 3·8 (s.d. 5·0) on nickel and nickel-free days respectively. The purpose was to make sure that the nickel was being taken. In all patients there was a rise when nickel was prescribed. Patients were assessed taking into account their own opinion and the assessor's opinion, which in all cases corresponded.

The statistical comparisons of placebo and oral nickel with regard to exacerbation of dermatitis must take into account the fact that each patient was assessed for both substances, i.e. there are pairs of results. In order to do this, fourfold tables (see Figs 1 and 2) were constructed to show the results for each substance for the group of patients—each dose of nickel being analysed separately against its corresponding placebo. On the null hypothesis that placebo and nickel had similar effects one would expect the following:

(a) Most patients to have either an exacerbation to both substances (+, +) or no exacerbation to either substance (–, –). It was found that twelve (55%) of the twenty-two patients had the

		Nickel 2·0 mg	
		+	–
Lactose	+	4	5
	–	5	8

FIGURE 1. Effect of nickel on hand dermatitis. Exacerbation (+), no exacerbation (–).

		Nickel 4·0 mg	
		+	−
Lactose	+	2	2
	−	6	12

FIGURE 2. Effect of nickel on hand dermatitis. Exacerbation (+), no exacerbation (−).

same reaction to placebo as they had to the 2 mg dose of nickel (Fig. 1); the corresponding figure for the 4 mg dose of nickel is fourteen (64%) out of twenty-two (Fig. 2).

(b) Those who reacted differently to placebo and nickel to divide approximately 50:50 into those who reacted to placebo but not to nickel and those who reacted to nickel but not to placebo. This hypothesis can be tested by McNemar's test when the numbers are sufficiently large or by the Binomial test if the numbers are small. Application of the appropriate test did not show any significant differences between placebo and nickel at the conventional level of $P<0{\cdot}05$. However, it must be remembered that the number of patients involved is small and that this could lead to a relatively insensitive statistical comparison.

These results suggest a tentative conclusion that there is no connection between the amount of nickel encountered in an ordinary diet and exacerbation of dermatitis. There is some suggestion in these results that a dose of nickel higher than in a diet likely to be encountered by most people will aggravate nickel dermatitis in some.

The current position then is that aggravation of hand dermatitis by dietary nickel is not proven, but in the opinion of these authors on the basis of this study a connection is unlikely. It is just possible that low-grade prolonged oral nickel could produce an aggravating effect without being detected on this test. It is very difficult to reduce nickel in the diet by much more than half so a low nickel diet is unlikely to help. The dietetic department of this hospital estimate that the low nickel diet which they provide has about 0·4 mg nickel and a normal diet in this area contains about 0·75 mg daily.

HIP PROSTHESIS AND NICKEL DERMATITIS

Hip replacement has become a very common operation. In the United States in 1976, 80,000 total hip replacements were carried out (Hori *et al.*, 1978). Further, a large percentage of women are allergic to nickel. In a study in this department of 340 nurses, ninety-eight gave a history of irritation from metal (earrings, bracelets, etc.). Of these, thirty-two gave a positive patch test so the following questions are important:

(1) Does a hip prosthesis sensitize?
(2) Does a prosthesis aggravate nickel dermatitis?
(3) Does nickel allergy predispose to hip rejection?
(4) Should all patients who are about to be subjected to hip prosthesis have a nickel patch test?

Various names are applied to hip prostheses: Charnley, McKee-Farrar, Miller, Thompson, Rings, Moore; these refer to the shape of the hip or socket. There are three main types of material used in the metal part of the hip (see Table 1): (1) stainless steel; (2) chrome/cobalt (vitallium); (3) titanium. Titanium hips do not contain chrome, cobalt or nickel.

The choice of material will depend on the strength or durability needed. Originally hip replacements were made of a metal head and cup and with these, wear particles of metal could be detected in solution around the joints (Swanson, Freeman & Heath, 1973), and chromium and cobalt levels in blood and urine were raised (Coleman, Herrington & Scales, 1973). There was also found to be a high incidence of metal sensitivity in failed prostheses: Benson *et al.* (1975) (28%), Elves *et al.* (1975) (38%), Munro-Ashman & Miller (1976) (46%), Deuterman *et al.* (1977) (13%). With metal on metal hip prostheses there was little doubt that the prosthesis could sensitize to nickel and this sensitization did appear to have a bearing on hip rejection. Now, however, a metal head and plastic acetabulum are used. The head is fixed deeply into the centre of the femur with methylmethacrylate which is polymerized from the monomer immediately before its insertion into the bone. The cup is fixed in the acetabulum in the same way with a few drill holes into the pelvic bone to hold it firm. Because metal is rubbing on plastic, the same levels of metal are not found in solution (Swanson *et al.*, 1973). There have been three studies of patients patch tested before and after hip replacement. The results are shown in Table 2.

TABLE 1.

	Stainless steel	Chrome cobalt (vitallium)
Carbon	0·03% maximum	0·35% maximum
Silicon	0·05% maximum	1·0% maximum
Manganese	0·5–2·0%	1·0% maximum
Nickel	10·0–14·0%	2·5% maximum
Chromium	16·50–18·00%	26·5–30·0%
Molybdenum	2·25–3·00%	4·5–7·0%
Sulphur	0·03% maximum	—
Phosphorus	0·04% maximum	—
Iron	Balance	1·0% maximum
Cobalt	—	Balance

TABLE 2.

Author	Number of patients	Positive patch test to nickel before hip replacement	Positive patch test to nickel after hip replacement
Carlsson, Magnusson & Moller, 1980	112	8·02%	8·9%
Rooker & Wilkinson, 1980	69	8·6%	1·4%
Deuterman *et al.*, 1977	173	5·8%	8·1%

These figures are strong evidence that present hip replacements do not sensitize to nickel and none of the patients with nickel sensitivity in any of these series had bother with loosening or rejection. Further, Rapaport (1980) found fifty consecutive patients with hip rejection all patch test negative to nickel and so 'there is no real proof that allergic reactions really cause loosening' (Lindberg, 1979). The evidence that modern hip prostheses which release little nickel into the tissues do not sensitize or aggravate dermatitis, and the evidence that prostheses which do release a lot of nickel into the circulation (older hip prostheses, heart valves, Smith-Peterson pins) (Lyell, Bain & Thomson, 1978; Oleffe & Wilmet, 1980; Elves *et al.*, 1975) do sensitize and aggravate dermatitis, tend to support the earlier tentative conclusion that large doses of nickel orally are necessary to aggravate nickel dermatitis.

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Neutron activation analysis of trace elements in skin. IX. Rubidium in normal skin

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SUMMARY

Regional variations of levels of rubidium (Rb) in normal adult skin were determined by non-destructive neutron activation analysis combined with gamma spectroscopy.

Rubidium has been classified as one of the trace elements with suspected biological function and unproven essentiality (Underwood, 1977). The earliest physiological study was made by Ringer (1882) who observed that the element affected the contractions of isolated frog's heart in a manner similar to potassium (K). Further biological studies have revealed a close physicochemical similarity between these two alkali metals which are of the same series as lithium (Li), sodium (Na) and caesium (Cs). This physicochemical similarity is not however reflected in their biological activities. An analogy may be drawn from two other alkali metals, K and Na. The former increases both respiration in muscle tissue and the rate of protein synthesis, whereas the latter has an inhibitory effect on both processes (Ei-Ichiro-Ochiai, 1977).

Experimental work on lower organisms, such as yeast (Lasnitzki & Szörényi, 1934) and bacteria (MacLeod & Snell, 1950), suggested that Rb might act as a nutritional substitute for K. A comprehensive study by Glendening, Schrenk & Parrish (1956) on 170 rats, showed that Rb has a much higher concentration in blood cells than in serum and that the kidney is the main path for its elimination. They concluded that trace quantities of Rb did not appear to be either a dietary essential or a stimulatory agent. However, experiments with monkeys (Carroll & Sharp, 1971) revealed that Rb produced antidepressant effects, contrasting with Li which reduced hyperactivity. Lithium is an established treatment in certain mental disorders, particularly mania and certain depressive states (Mendels, 1973; Goodwin & Ebert, 1973). Despite the widespread use of Li compounds, both therapeutically and prophylactically, their mode of action is still poorly understood. It was suggested by Wiles (1978) that lithium might displace Na, K, Mg or Ca from enzyme sites.

Patients with psoriasis and bipolar affective illness have shown marked deterioration of their skin condition when treated with lithium (Carter, 1972; Skott, Mobacken & Starmark, 1977).

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Aiking & Tempest (1977) demonstrated that only Rb could satisfactorily substitute for K in supporting the growth of *Candida utilis* in a defined simple salts medium in a chemostat culture.

The total human body content of Rb is 0·32 g (ICRP, 1975; Venugopal & Luckey, 1978) and the average intake for man is about 2 mg/day, mainly through the diet (Soman *et al.*, 1969). Rubidium has been determined in human blood and urine (Wood, 1969), in brain (Höck *et al.*, 1975), in liver (Lievens *et al.*, 1977) and in other tissues (Persigehl *et al.*, 1977).

Little attention has been paid to levels of Rb in human skin and very few estimations are available. Molin & Wester (1976) measured Rb in the epidermis of nine subjects from the middle trunk and found levels of 9·1 ± 4 p.p.m. (mean ± s.d.). Persigehl *et al.* (1977) found that Rb decreased in the skin with increasing age in twenty-one subjects.

The purpose of our study was to determine the epidermal and dermal concentrations of Rb in two skin regions and to compare its distribution to that of elements of proven essentiality to man.

MATERIALS AND METHODS

Samples of skin were obtained from adult cadavers free of skin disease. The epidermis was separated by the steam method (Molokhia & Portnoy, 1969). Specimens of liver were obtained from cadavers free from either skin or liver diseases; serum and hair samples were obtained from healthy adult volunteers. Samples were dried at 50°C for several days. Reference materials (orchard leaves, bovine liver) were prepared as recommended by the National Bureau of Standards (NBS), and small amounts of approximately 200 mg were sealed in clean silica ampoules. The certified value of Rb content in these materials was used to calculate Rb concentration in skin samples. Samples of abdominal wall skin weighing 10–50 mg together with the two standards were irradiated at Harwell at a thermal neutron flux of 6×10^{12} neutrons cm^{-2} s^{-1} for 1 week. Samples of plantar skin weighing between 50–150 mg were irradiated in the Universities Research Reactor, Risley, at a thermal neutron flux of about 2×10^{12} neutrons cm^{-2} s^{-1} for 3 weeks*. Great care was taken to avoid contamination during sample collection and treatment. All samples and standards were subjected to a post-irradiation cooling period of 2–4 weeks. Gamma ray spectra were measured by a shielded 90 cm^3 co-axial germanium lithium Ge(Li) detector (resolution 2·1 keV at 1332·4 keV of ^{60}Co) connected to 4096 channel pulse height analyzer. Samples and standards were counted for 4,000 s at least twice during decay. Analysis of the spectra was performed using a computer programme, and the area of the ^{86}Rb peak at 1076·6 keV was corrected for decay where necessary. Rubidium concentration was obtained for each sample in μg/g dry weight.

The reaction used for the determination of Rb by neutron activation was $^{85}Rb\,(n,\gamma)\,^{86}Rb$. The parent nuclide has an abundance of 72·15% and the isotopic thermal neutron activation and absorption cross-section is 0·4 barn.

RESULTS

Table 1 shows the concentrations of Rb in epidermis and dermis of the comparable cutis from twenty-four autopsy specimens of abdominal wall skin. The Rb concentration of the epidermis was found to be higher than that of the dermis and the difference in the means is highly significant ($P < 0{\cdot}001$).

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* For readers not familiar with the method of neutron activation analysis it should be pointed out that these apparently different treatments of skin samples from abdominal wall and from foot were entirely comparable (see Bowen, 1975).

TABLE 1. Rubidium concentration in paired samples of abdominal epidermis and dermis ($\mu g\ g^{-1}$ dry weight)

	No. of samples	Range	Mean	± s.d.	Median
Epidermis	24	2·34–10·95	6·62	2·32	6·30
Dermis	24	1·07 7·85	3·47	1·43	2·90

P (two-tailed t-test) < 0·001.

Table 2 shows rubidium concentration of the epidermis and dermis in ten plantar skin samples. It is interesting to note that the rubidium concentration in the epidermis of this region is lower than that of the dermis and the difference between the means is highly significant ($P < 0·001$). There was no difference between males and females in the rubidium concentration in the skin.

It was found that rubidium in the epidermis and dermis decreased with increasing age. The correlation coefficient was −0·652 ($P < 0·01$) for the epidermis and −0·353 ($P < 0·05$) for the dermis. The correlation coefficient between Rb in the dermis and Rb in the epidermis was calculated and found to be 0·5342 ($P < 0·01$).

The concentration of rubidium in the skin as shown in Tables 1 and 2 was tested for normality and the results are shown in Figs 1 and 2. The observed frequency distribution was represented by the histogram in which a superimposed normal distribution fitting based on a chi-square (χ^2) test for the goodness of fit was indicated by letter 'n': χ^2 (epidermis) = 1·635, which with d.f. = 2 is not significant. χ^2 (dermis) = 7·383, which with d.f. = 3 is not significant. Therefore, the observed frequency distributions of Rb in both epidermis and dermis do not differ significantly from the normal distribution. Table 3 presents levels of Rb in human blood (serum), scalp hair

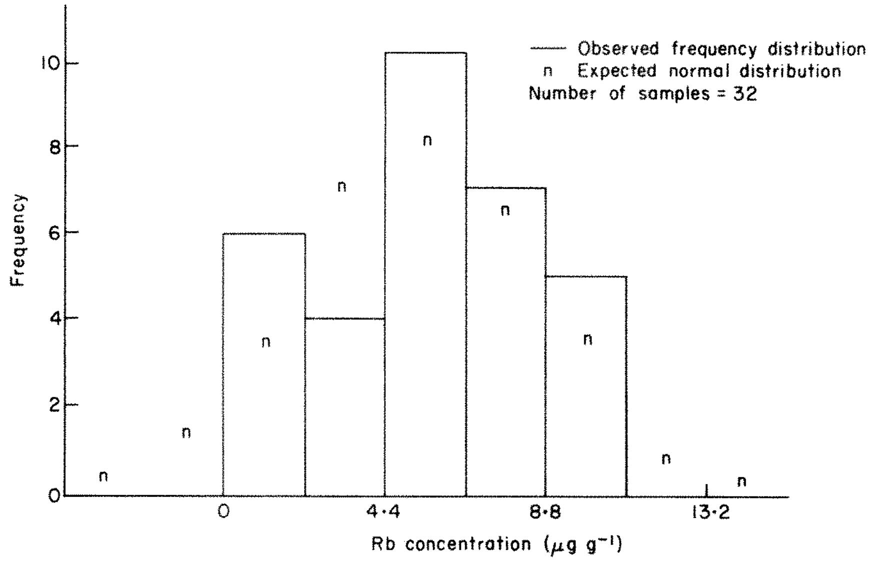

FIGURE 1. Rubidium distribution in abdominal epidermis ($\mu g\ g^{-1}$ dry weight).

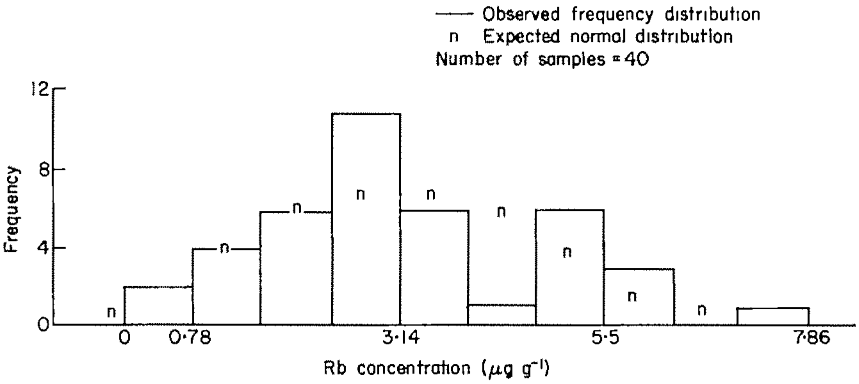

FIGURE 2. Rubidium distribution in abdominal dermis ($\mu g\ g^{-1}$ dry weight).

TABLE 2. Rubidium concentration in plantar skin ($\mu g\ g^{-1}$ dry weight)

	No. of samples	Range	Mean	± s.d.	Median
Epidermis	10	0·82–3·46	1·80	0·70	1·65
Dermis	10	3·56–5·87	4·62	0·84	4·46

P (two-tailed t-test) < 0·001.

TABLE 3. Rubidium concentration in three human tissues ($\mu g\ g^{-1}$ dry weight)

	No. of samples	Range	Mean	± s.d.
Serum	18	2·100–6·87	3·393	1·171
Hair	6	0·27–1·91	0·863	0·738
Liver	30	8·77–34·25	21·158	6·52

and liver, the latter showing the highest levels among the three tissues studied. This table is given for comparison.

DISCUSSION

The accuracy and precision of the method used in this study have been tested by repeated analyses of orchard leaves standard, the value obtained for Rb being 13·36 ± 0·5 p.p.m. (mean ± s.d.) compared with the certified value of 12·00 ± 1 p.p.m. (mean ± s.d.).

The element concentration in the abdominal epidermis and the decrease with aging compared well with some recently published results (Molin & Wester, 1976; Persigehl *et al.*, 1977).

Alkali metals such as Na and K are required for the optimum activity of the enzyme ATPase.

The ATPase was found in membranes with a sodium pump (Ei-Ichiro-Ochiai, 1977) and is referred to as Na/K-activated ATPase. Potassium (K) can be replaced by other cations such as Rb and Cs.

Phospholipid bilayer membranes are virtually impermeable to K or Na. It was found however that, when a small amount of an antibiotic (valinomycin) was introduced into the system, the resistance fell by five orders of magnitude and the permeability of the membrane to K rose by the same amount (Ei-Ichiro-Ochiai, 1977).

The stability constant of Rb in valinomycin at 25°C in methyl alcohol was found to be the highest for cation-carrying antibiotic complexes. Valinomycin is a cyclic oligopeptide with twelve carbonyl groups, which are potential metal coordinators, and a number of hydrophobic branching groups. It has been demonstrated that valinomycin can coordinate to K and the resulting K complex, although charged, can diffuse readily through the hydrophobic interior of the membrane. It is therefore possible that valinomycin, or other similar antibiotics, can coordinate with Rb as it does with K to form a complex which will then diffuse through the skin resulting in a therapeutic effect. Further research on this aspect would be of interest.

Until now, it has not been possible to demonstrate the essentiality of Rb. However, recent studies show that Rb may be counted among the essential elements (Höck *et al.*, 1975). Indeed Siegers *et al.* (1977) suggested that Rb concentration in rats is genetically controlled and cannot be explained by differing nutritional or other environmental conditions.

The present study showed that Rb in skin and other tissues behaved like an essential trace element.

Further hybridization experiments should help in deciding if Rb concentrations are indeed under genetic control and may certify the essentiality of Rb.

Whether Rb could be substituted by potassium in the nutrition of man and animals is still a matter requiring further investigation.

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Sclerosing lipogranuloma of the male genitalia: ultrastructural study

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SUMMARY

Ultrastructural study of a case of sclerosing lipogranuloma showed a predominantly histiocytic-like cell infiltrate with numerous intracytoplasmic vacuoles of varied sizes without any limiting membrane and located in the deep dermis and subcutaneous fat. Fibroblasts appeared normal. Polymorphonuclear cells and macrophages were absent. This appearance was different from that described in 'classical' phagocytosis, mucopolysaccharidoses or polyvinylpyrrolidone storage syndrome.

Lipogranuloma affecting the male genitalia is a rare condition which is considered to be of factitial origin induced by injection of exogenous material such as oil, paraffin or silicone (O'Rourke, 1967; Carlson, 1968; Arthaud, 1973; Engelman, Herr & Ravera, 1974). Clinically it may masquerade as a malignant tumour and inguinal lymph nodes may be enlarged. There is no sign of local inflammation. The histological features are characteristic with a granulomatous inflammation and a Swiss cheese-like appearance.

We report the ultrastructural study of such a case.

CASE REPORT

A 52-year-old bachelor with a history of infantile encephalitis and consequent deafness and partial dumbness was first seen in May 1980 because of urinary discomfort. Physical examination showed a diffuse thickening of the skin due to deep-seated confluent nodules (2 to 4 cm in diamter) located symmetrically on the pubic area, scrotum and shaft of penis (Fig. 1). The glans penis was entirely covered by the nodular masses. The overlying skin had a normal appearance. No signs of inflammation or systemic symptoms were noted. The inguinal lymph nodes were not enlarged. No abnormalities were detected in the routine laboratory tests. Erythrocyte sedimentation rate and blood counts were normal. X-rays of the pelvis and upper femurs showed no evidence of calcification in the genital area. An intravenous pyelogram was normal. Serological tests for syphilis were negative. Information on duration was not given. The patient said he had no 'big problems' and saw no relationship of psycho-

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logical factors to his illness. He denied knowledge of any injection, but remembered trauma to the genitalia without any wound which occurred in 1962.

Microscopically, the deep dermis and subcutaneous fat were occupied by a granulomatous inflammation (mainly lymphocytes and histiocytes) with marked sclerosis (Fig. 2). Under low magnification numerous vacuoles of varied size and shape were observed. The empty spaces were without any cellular lining or intraluminal residue. In some areas, fat cells with histiocyte-like appearance and granular cytoplasm were found. No vasculitis was found. Sections from frozen tissue stained with ORO showed that the vacuoles contained fat-positive substances. Little birefringent material was seen. No infrared-absorption spectrophotometry was performed.

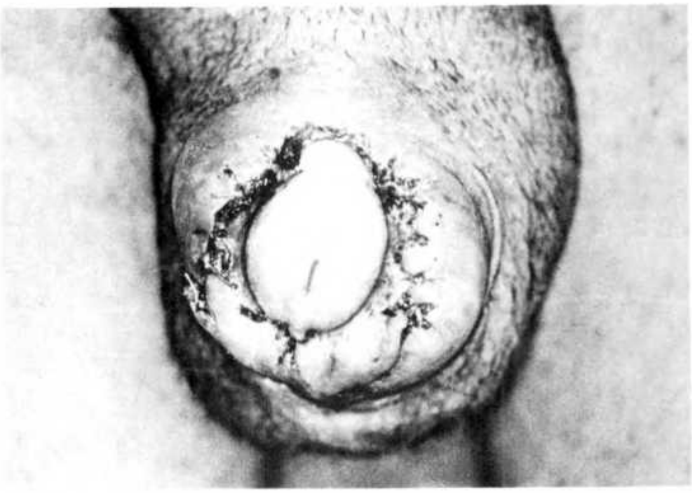

FIGURE 1. Sclerosing lipogranuloma of penis and scrotum.

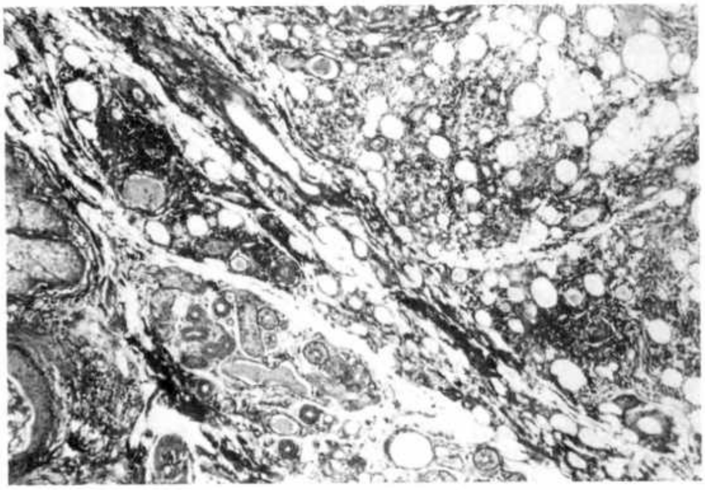

FIGURE 2. Vacuoles of various sizes (× 20).

Ultrastructurally, most of the cells contained vacuolar intracytoplasmic inclusions. When the vacuoles were small, organelles were normally present and the cells were considered to be histiocytes. Near large vacuoles, the nuclei were elongated and the architecture of the cells was no longer recognizable. These vacuoles were usually without any limiting membrane but in some instances, electron-lucent vacuoles seemed to have a unit-limiting membrane. Dense osmiophilic bodies were noted in close proximity to lipid-like vacuoles. No lamellar myelin figures were found (Figs 3 and 4). Scattered

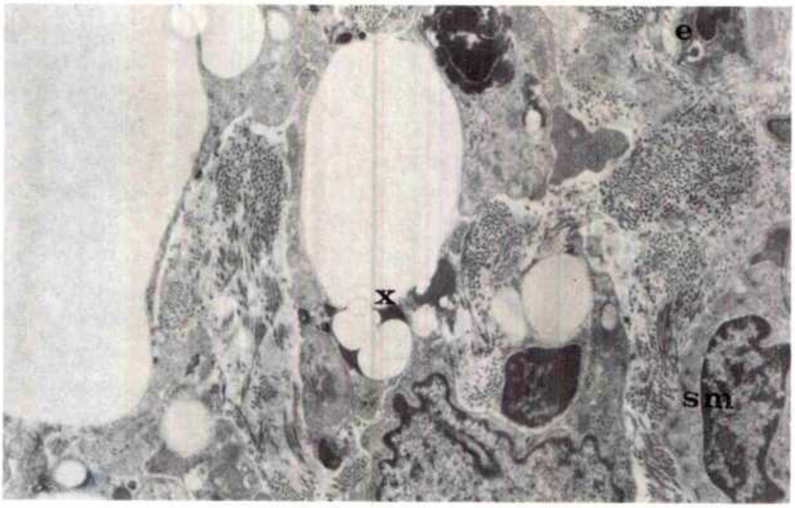

FIGURE 3. Large vacuole in the cytoplasm of histiocyte-like cell and numerous small sized vacuoles. Absence of extracellular electron-lucent vacuoles. Note the presence of dense osmiophilic bodies in the vicinity of vacuoles (x). Normal appearance of endothelial cell (e), smooth muscle cell (sm) and vascular basement membrane. (× 7,000).

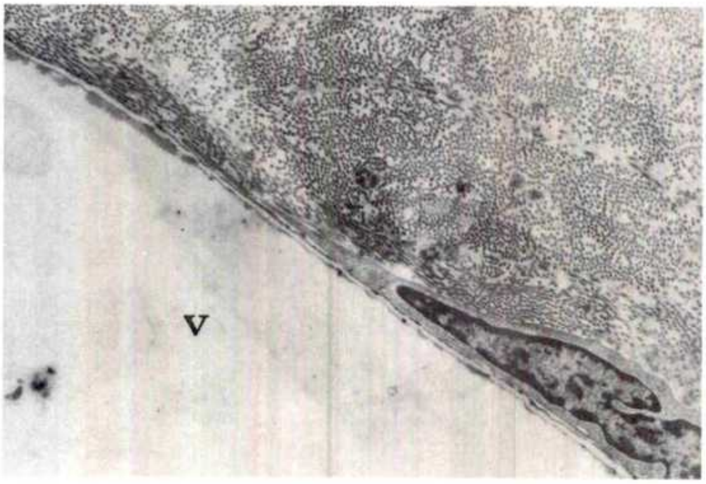

FIGURE 4. Elongated histiocyte-like cell near large vacuole (V). Lining of the vacuole interpreted as an artifact. (× 7,000).

lymphocytes with a normal appearance were observed in the vicinity of vacuolated cells. Polynucleated cells were absent. The macrophages and fibroblasts were not involved in the engulfing process. Collagen fibres and endothelial cells appeared normal.

COMMENT

Injection of foreign lipid material into the skin has been known for centuries. Benedek (1913) first used the term 'paraffinoma'. Therapeutic and/or cosmetic use of oil, paraffin and silicone has now been condemned owing to its severe damaging effect (Greaney & Jackson, 1977) and possible cancer hazard (Colomb, 1962). Injection of oil or paraffin into the male genitalia to increase their size or facilitate erection is a well known, although rare, phenomenon (Quénu & Perol, 1947; Smetana & Bernhard, 1950; Oertel & Johnson, 1977). Most patients deny having had such injections. Contusions and traumas are often advocated. The discovery of severe psychological disturbances may help to confirm the diagnosis (Forstrom & Winkelmann, 1974). Smetana & Bernhard (1950) coined the term 'sclerosing lipogranuloma' for a lesion of the male genitalia believed to be 'a local reactive process following injury to adipose tissue'. The histological appearance of this case was indistinguishable from that of a paraffinoma. It seems now admissible to define this entity after Oertel and Jolnson's interpretation as 'a local reactive process following injection of exogenous lipids into the subcutaneous tissues'. The discovery of paraffin hydrocarbons by infrared spectrophotometry in such cases ruled out the possibility that these lesions might be interpreted as a response to the degradation of endogenous lipids (Kumagai, Abe & Shiokawa, 1979; Oertel & Johnson, 1977). No evidence for ruptured dermoid cysts and subsequent reaction to endogenous lipids was noted in our case. The clinical and histological aspects were also different from those of localized scleroderma (Kumagai *et al.*, 1979). No calcium deposits were seen (paraffin and silicone do not undergo calcification themselves—Wren & Crosbie, 1968). Since the lesions were radiolucent, they may be due to the injection of a paraffin or oil compound rather than to silicone which is more radio-opaque (Koide & Katayama, 1979).

In our study, the electron micrographs showed histiocyte-like cells containing large electron-lucent vacuoles within their cytoplasm. The vacuoles, usually lacking a cellular lining, appeared sometimes surrounded by a unit-limiting membrane (Fig. 4). We interpreted this peculiar aspect as an artifact due to the retraction of the plasma membrane of the cell during the processing of the tissue. The dense osmiophilic bodies found in the vicinity of the vacuoles (Fig. 3) were assumed to be primary lysosomes rather than stored lipids. No secondary lysosomes were found. This appearance was different from that of 'classical' phagocytosis, faulty degradation of endogenous substances or the polyvinylpyrrolidone storage syndrome where lysosomes are primarily involved. No lamellar or pseudomyelinic structures identical to those described in various lipidoses were noted (Lasser, Carter & Mahoney, 1975). Fibroblasts were not involved in the vacuolisation process as they are in mucopolysaccharidoses (Bioulac *et al.*, 1975). No striking changes in the morphology of the cells were suggestive of lymphangioma or liposarcoma.

Ultrastructural study may prove to be a useful method for confirming the diagnosis of sclerosing lipogranuloma.

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Pharmacology and Treatment

The relief of pruritus in primary biliary cirrhosis by hydroxyethylrutosides

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SUMMARY

Hydroxyethylrutosides (Paroven, Zyma) was effective in relieving chronic pruritus in six patients with primary biliary cirrhosis.

Intractable generalized pruritus is a common and distressing feature of primary biliary cirrhosis (PBC) and is usually attributed to accumulation of bile salts in skin (Kirby, Heaton & Burton, 1974; Schoenfield, Sjovall & Perman, 1967), although recent work has cast some doubt on this (Ghent, Bloomer & Klatskin, 1977; Lauterburg *et al.*, 1980). The usual treatment is oral cholestyramine which is assumed to act by binding bile salts in the intestinal lumen, thus gradually leaching them from the skin. However, many patients find it unpalatable and it has the major disadvantage of reducing further the intraluminal concentration of bile salts in the gut, aggravating steatorrhoea and fat-soluble vitamin deficiency (Compston & Horton, 1978; West & Lloyd, 1975). An antipruritic agent with a different mode of action would be valuable and we have conducted a small pilot study to examine the effect of hydroxyethylrutosides (HR) in this disease.

PATIENTS

Six patients with typical PBC, confirmed in all cases by liver biopsy and the presence of circulating antimitochondrial antibody in high titre, were studied. All gave informed consent.

Case 1

A 30-year-old woman who had experienced generalized pruritus for two and a half years presented with a history of 6 weeks jaundice. Investigation revealed that she had PBC. She was given HR in a dose of 250 mg t.d.s. reducing over three weeks to 250 mg daily. No other medication was given and pruritus was completely relieved. During treatment bilirubin levels fluctuated between 35 and 56 μmol/1. During subsequent penicillamine therapy itching returned and was again controlled by reintroducing HR in a single daily dose of 250 mg.

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Case 2

A 26-year-old lady, proved in 1972 to have PBC began to itch one year later. She became asymptomatic after taking HR 250 mg t.d.s., reducing over three weeks to 250 mg daily. Maintenance therapy has proved effective for more than 3 months during which the degree of cholestasis has been unchanged with plasma bilirubin levels between 100 and 110 μmol/l.

Case 3

A 44-year-old female with a 5-year history of pruritus was shown to have PBC 2 years ago. Cholestyramine 4 g.b.d. failed to control her itching which was rapidly relieved by HR 250 mg t.d.s. In this patient lower doses of HR were not effective and added cholestyramine was necessary if the daily dose of HR was reduced below 750 mg. During treatment plasma bilirubin levels were stable at between 134 and 146 μmol/l.

Case 4

A 64-year-old woman developed jaundice and pruritus 3 years ago, shown to be due to PBC. The itching was relieved by cholestyramine 4 g. t.d.s. but she developed steatorrhoea (faecal fat 65 mmol/day) on treatment with the drug. It was substituted by HR 250 mg q.d.s. and her diarrhoea improved, without a return of the pruritus. Substitution of the HR by an identical placebo led to a recurrence of itching which subsided again with reintroduction of HR. Plasma bilirubin levels during the study ranged between 80 and 93 μmol/l.

Case 5

A 60-year-old lady developed PBC in 1977. At presentation she was experiencing mild pruritus which responded well to cholestyramine 4 g daily. One year ago cholestasis markedly worsened, itching reappeared and was not controlled by cholestyramine 4 g t.d.s. However, HR 500 mg q.d.s. dramatically abolished her pruritus and the cholestyramine could be stopped. Again substitution of HR by and identical placebo resulted in a rapid return of her symptoms, relieved by reintroducing HR. Plasma bilirubin levels remained unchanged at 250 μmol/l.

Case 6

A 61-year-old female presented 4 years ago with jaundice, itching and weight loss. Investigations confirmed PBC. She was given chlorpheniramine 4 mg t.d.s. and 8 mg at night which relieved her itching but left her with a constant sense of tiredness. She was given HR 400 mg q.d.s. reducing after 1 week to 250 mg q.d.s., and it was then possible to withdraw the chlorpheniramine without recurrence of the pruritus and with improvement in her alertness. Bilirubin levels varied during this time between 102 and 120 μmol/l.

DISCUSSION

The principal clinical use of HR is in the treatment of the symptoms of venous insufficiency in the legs. It decreases capillary permeability to crystalloid and we have shown that it reduces the increased transvascular leakage of albumin in cirrhosis (Hishon, Hunter & Rose, 1980). During these studies the incidental observation that HR relieved itching in PBC was made and this led to the present work.

Although pruritus is a markedly subjective symptom we believe our results are not due to a simple placebo effect and the observations in the two patients given dummy tablets support this. The absence of any change in conventional liver function tests during treatment makes a spontaneous or drug-induced alteration in disease activity unlikely as an explanation, but the biliary system is the major route of excretion of HR (Griffiths, 1980), and enhanced bile salt secretion cannot be excluded. In our patients the beneficial effect of HR on pruritus may have been mediated therefore by prevention of bile leakage from dermal blood vessels although we have no direct evidence for this.

No patient experienced any side effects from HR and in particular there was no worsening of diarrhoea. All patients found the drug simple and palatable to take. A larger controlled trial seems warranted.

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A severe 'stasis eczema', associated with low plasma zinc, treated successfully with oral zinc

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SUMMARY

We describe a case of non-ulcerating severe stasis eczema in an elderly female associated with a low plasma zinc and which responded to treatment with oral supplements. The dermatological changes were not accompanied by oedema and were most marked over the lower legs and ankles, with patchy erythematous lesions extending to the inner thighs, over the dorsal surface of both hands and the forearms. There was some excoriation and a degree of lichenification. These lesions were intensely pruritic and resolved almost completely following treatment with oral zinc sulphate (220 mg daily) on two separate occasions. Histological appearances of biopsy material included those of stasis eczema but there was also a marked degree of parakeratosis.

The cause of the low plasma zinc was not identified.

The clinical associations between abnormalities of zinc metabolism and dermatological complications are well known and have recently been reviewed (Weismann, 1980). Most attention has been directed towards acrodermatitis enteropathica and its treatment with zinc but low plasma concentrations are also found after prolonged intravenous feeding (Kay *et al.*, 1976), hepatic cirrhosis, malabsorption (Walker *et al.*, 1973; Weismann *et al.*, 1978), kwashiorkor (Golden, Golden & Jackson, 1980) and in a series of apparently unrelated conditions such as infectious disease, pernicious anaemia, malignancy, leukaemia, rheumatoid arthritis, jaundice and renal disease.

A low plasma zinc concentration has been noted in stasis ulceration of the legs (Greaves & Boyde, 1967; Withers *et al.*, 1968; Hallböök & Lanner, 1972; Greaves & Skillen, 1970) and for unknown reasons these patients appear to have a slightly but significantly shortened biological half life of zinc as determined by administration of ^{65}Zn followed by whole body counting (Hawkins & Marks, 1976). Oral zinc, however, is not necessarily an effective treatment (Greaves & Ive, 1972). No report as far as we know describes the intensely pruritic eczematous 'stasis' changes, easily reversible with oral zinc, which were seen in this elderly lady.

CASE REPORT

During hospital admission an 82-year-old Caucasian woman suffered a brain stem ischaemic episode

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with transient loss of consciousness resulting in urinary and faecal incontinence for several months. She has a past history of myocardial infarction and a successful anterior resection of the sigmoid colon for carcinoma 10 years previously. There was no evidence of excess phytate ingestion, chronic alcohol abuse, renal disease, diabetes, malabsorption, rheumatoid arthritis, collagen disease or recurrence of malignant disease.

During her admission she was treated intermittently for mild heart failure (digoxin, spironolactone and hydroflumethiazide—Aldactide®) and over 3 months complained increasingly of an extremely itchy confluent erythematous and excoriated rash with lichenification on both legs (Fig. 1), and to a lesser extent on both forearms and the backs of both hands with patchy lesions seen on the upper legs, knees and inner thighs. She was clearly depressed, poorly motivated and had some considerable difficulty in walking. There was no evidence of hepatosplenomegaly or abnormally sparse hair growth.

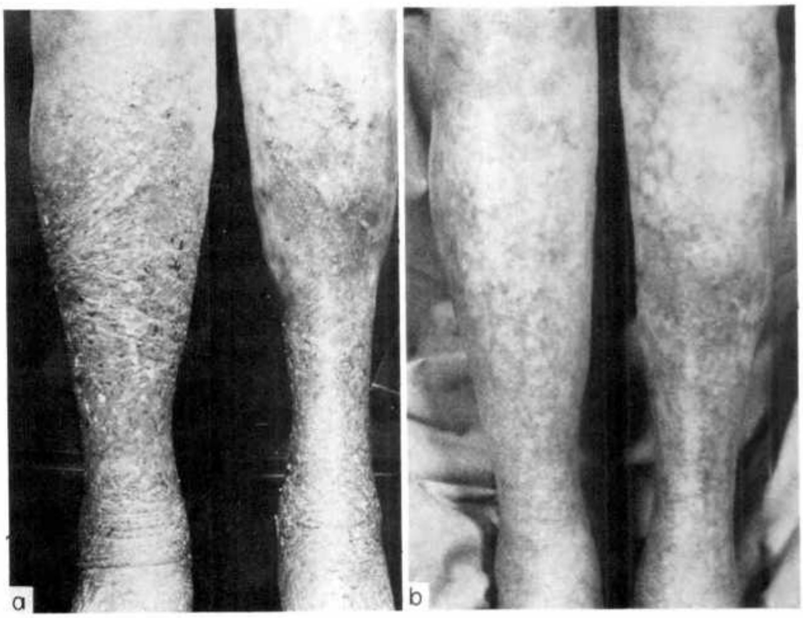

FIGURE 1. Dermatological appearances (a) before and (b) after 8 weeks treatment with oral zinc.

Results of investigations were essentially unremarkable with values within the normal range for the following: haemoglobin (16·0 g/l), red cell indices, white cell count, serum B_{12}, red cell folate, blood sugar, plasma urea, electrolytes, calcium, magnesium (0·71 mmol/l), phosphorus, albumin (42 g/l), globulin (29 g/l), protein electrophoresis, liver function tests and xylose absorption test. Iron (5·0 μmol/l) and total iron binding capacity (38 μmol/l) were slightly below the normal range and the non-fasting 10 a.m. plasma zinc concentration was 6·7 μmol/l (normal range: 11·4–18·4 μmol/l). A chest X-ray and barium enema were both normal.

The subnormal plasma zinc concentration as determined by atomic absorption spectrometry (Davies, Musa & Dormandy, 1968) was investigated further with a zinc loading test performed as described by Pécoud, Donzel & Schelling (1975). A large pharmacological dose (220 mg $ZnSO_4\ 7H_2O$) was given when fasting and blood was then drawn for plasma determinations at intervals over 6 h. The response was essentially normal with a rise in plasma concentration to a maximum of 18·5 μmol/l (normal range 28·3 ± 6·5 μmol/l, mean ± 1 s.d.).

Treatment with oral zinc (220 mg $ZnSO_4$ $7H_2O$ daily) was started, and within 1 week her itching had disappeared. Within 4 weeks the rash had largely resolved, by which time the plasma zinc had reached 17·8 μmol/l. It was also considered that there was considerable improvement in her mental state. She appeared to gain strength and her ataxic gait resolved.

Stopping zinc therapy after 2 months resulted in a return of the itching within 6 weeks (plasma zinc 11·2 μmol/l) and the rash on the legs and both hands in 10 weeks (plasma zinc 10·4 μmol/l). The rash was biopsied and light microscopy showed appearances compatible with stasis eczema and a marked degree of parakeratosis. The zinc content of the affected skin was, however, normal. Oral zinc (220 mg daily) was restarted and again within two weeks the rash disappeared as the plasma zinc rose to 25·2 μmol/l.

DISCUSSION

The patient suffered intense pruritus, erythema and lichenification which seemed excessive for what had been confidently diagnosed as stasis eczema with autosensitization of the hands. It was these features together with the specific involvement of the extremities and the age of the patient that prompted determination of the plasma zinc. The skin manifestations were unlike the oral and perioral vesicopustular eczema and alopecia seen following long-term intravenous feeding (Kay *et al.*, 1976; Weismann, 1980) where the plasma zinc concentration was very much lower and the situation more acute. They did resemble, however, verrucose or psoriasiform patches on the extremities with eczema craquelé (asteatosis) seen in more chronic zinc deficiency (Weismann *et al.*, 1978). A reddish brown erythema of the finger creases, considered an important diagnostic feature, was not seen (Weismann, Wanscher & Knudsen, 1977). In common with other patients (Prasad *et al.*, 1963) this woman was poorly motivated, apathetic and depressed, and it was not until treatment had been started with substantial improvement in these features and her ataxic gait that their significance was realized. The observed dermatological changes which included marked parakeratosis occurred in the presence of a normal value for total skin zinc but a dissociation between plasma and skin concentrations is well known (Oon *et al.*, 1974). It is worth observing that local anaesthetics such as lignocaine (Xylocaine) can contain significant quantities of zinc whereas mepivacaine (Carbocaine) and procaine (Novocain) are reported to contain much less (Parkinson, Millikan & Anderson, 1979).

The claimed association between the skin changes and zinc status is based on two successful therapeutic trials of oral zinc where relief of the pruritus (spontaneously commented upon by the patient within 2 days of starting treatment) preceded improvement of the eczema. The time taken for the latter to occur (2–3 weeks) is similar in other reports (Kay *et al.*, 1976; Weismann, 1979). Topical steroid, given for 2 days in general practice, produced substantial relief and in this context may be relevant in that zinc stabilizes lysosomes *in vitro* (Chvapil, Ryan & Zukoski, 1972). The features we describe are not included in recent reviews of symptoms associated with hypozincaemia (Aggett & Harries, 1979; Prasad, 1979; Weismann, 1980).

The cause of the low plasma zinc was not determined. The patient did not suffer from any condition normally associated with a low plasma value except possibly chronic illness (Greaves & Boyde, 1967) and there was no history of a diet high in phytic acid (unrefined grain and legumes). The most likely explanation seemed to be socio-economic in that her intake of animal products was moderate and low protein diets have been associated with low zinc levels (Mansouri, Halsted & Gombos, 1970).

In conclusion we suggest that elderly or deprived patients suffering from stasis eczema resistant to conventional treatment (especially if accompanied by marked pruritus and erythema) should have their zinc status determined. If subnormal values are found then oral supplements would appear to be indicated.

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Azathioprine therapy in the management of persistent erythema multiforme

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SUMMARY

Two cases of recurrent erythema multiforme of unknown aetiology are reported. The term 'persistent' is appropriate as the lesions recurred whenever steroid therapy was discontinued. Both patients were eventually controlled with azathioprine alone, and one patient required treatment for 3 years.

Erythema multiforme (EM) is generally regarded as a self-limiting process. Acute attacks usually resolve within 3–6 weeks, and even patients with recurrent EM are free of lesions for long periods between each episode. Occasionally, however, attacks occur with such frequency that lesions may persist from one episode to the next and such cases present a considerable management problem. We report two such cases satisfactorily controlled by azathioprine therapy.

CASE I

A 44-year-old female patient of West Indian extraction presented in 1973 with a painful blistering eruption. Examination revealed numerous large target lesions up to 2 cm in diameter, many of which were surmounted centrally by haemorrhagic bullae. Oral ulceration was also present. A clinical diagnosis of bullous erythema multiforme was confirmed on biopsy. Direct and indirect immunofluorescence studies were negative.

Investigations did not reveal any cause for the attack, and the patient responded to treatment with prednisolone 30 mg daily. When her dosage was reduced her condition promptly relapsed and further investigations were carried out to exclude an underlying connective tissue disorder. Apart from a weakly positive ANF and a slightly raised DNA binding of 30 units/ml, there was no other evidence to support a diagnosis of SLE. No cryoglobulins were detected in the serum, though more specific tests for immune complex detection were not carried out. During the course of the next 2 years the patient required maintenance therapy with oral steroids. Azathioprine was added to her regime, and on a dosage of 100 mg daily, oral steroid therapy was eventually discontinued. The patient required treatment with azathioprine for a further three years before it was possible to stop the azathioprine without a recurrence of her EM.

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CASE 2

A 19-year-old Arabian student presented in 1978 with a 4-year history of a recurrent blistering eruption affecting the limbs. Examination revealed numerous target lesions with oral ulceration, and a diagnosis of bullous erythema multiforme was confirmed on biopsy. Direct immunofluorescence was negative, routine laboratory tests were normal, and no precipitating factors were identified from the history or from the laboratory investigations.

In 1980 the patient was re-admitted as the attacks were recurring with increasing frequency and were not controlled by weekly injections of Synacthen Depot. Periods of remission were lasting for 1–2 weeks only. The following immunological investigations were performed and were within normal limits: peripheral B and T cell counts, total haemolytic complement, C_3 and C_4 levels, and total alternate pathway activity.

Immune complexes were not detected by Clq and monoclonal rheumatoid factor binding assays, but a mixed cryoglobulin was present at a concentration of 155 μg/ml serum. The patient's therapy was changed to azathioprine 150 mg daily which was continued for 3 months. During this period he suffered no further attacks of EM and has remained in remission for 6 months since therapy was discontinued. One month after starting therapy the level of cryoglobulin had fallen to 65 μg/ml but it subsequently rose to 177 μg/ml without a recurrence of disease activity.

DISCUSSION

Although precipitating factors are often identified in EM, half the cases are of unknown aetiology (Tonnesen & Soter, 1979). Recurrent cases of EM may be associated with recurrent herpes simplex, but no such infective agent was identified in either of our two cases. Although the immunopathogenesis of EM is poorly understood several lines of evidence point towards a Type III hypersensitivity reaction. A variety of techniques, including cryoprecipitation, have been used to demonstrate immune complexes in the serum of patients with EM (Swinehart *et al.*, 1978; Imamura *et al.*, 1980; Huff, Weston & Carr, 1980; Wuepper, Watson & Kazmierowski, 1980; Bushknell, Mackel & Jordon, 1980). Elevated levels of immune complexes and reduced levels of complement have been detected in blister fluid from patients with bullous EM and immunofluorescence studies have shown deposition of immunoglobulin and complement in lesional skin (Kazmierowski & Wuepper, 1978; Imamura *et al.*, 1980; Bushknell *et al.*, 1980; Huff *et al.*, 1980). The finding of cryoimmunoglobulins in our second case is consistent with an immune complex pathogenesis. However, it is difficult to attribute the beneficial effect of azathioprine to its effect on the level of circulating cryoglobulin, since an initial fall was followed by a return to the original level without a recrudescence of disease activity. Experimental studies which have sought to determine the selectivity of immunosuppressive therapy on humoral and cell mediated immune responses indicate that azathioprine causes equivalent inhibition of both T and B cell function (Otterness & Chang, 1976). This lack of selectivity prevents any useful conclusions being drawn about its beneficial effect in cases of erythema multiforme.

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Case Reports

An unusual presentation of systemic amyloidosis

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SUMMARY

We report a case of systemic amyloidosis presenting as intracutaneous haemorrhage following cardiac arrest.

CASE REPORT

E.G., a 69-year-old man in otherwise good health and with an unblemished skin history attended the urological out-patient department in November 1979 with a 3-month history of painless haematuria. Mild prostatic hypertrophy was recorded on rectal examination but the remainder of the physical examination, the full blood count and the intravenous urogram were normal. When admitted for cystoscopy several weeks later he had developed signs of right-sided heart failure with minimal cardiac enlargement radiologically. The oedema had not been diminished by standard doses of digoxin and a diuretic. Before general anaesthesia, induced by thiopentone and halothane, physical manipulation from his bed to a trolley was accompanied by the appearance of petechial haemorrhages on the chest wall and a prophetic theatre note, 'skin very fragile with epithelial separation on minimal finger trauma'.

Cystoscopy under general anaesthesia showed no abnormality but soon after endoscopy he had a cardio-respiratory arrest triggered by a profound ventricular bradycardia. A brisk recovery followed artificial ventilation, external cardiac massage and the appropriate intravenous therapy, and this was accompanied by the appearance of peri-orbital ecchymoses, haemorrhage under the tarsal conjunctivae and separation, with digitate extensions, of the skin over the sternum at the site of the external cardiac massage. The coagulation screen, platelet count and total protein fractions were normal at this time. In spite of the appropriate therapy his cardiac failure rapidly progressed and a further episode of cardiac arrhythmia was the terminal event.

Ante-mortem skin biopsy of the chest wall showed deposits of amyloid around the cutaneous appendages and in the papillary dermis. Blistered and eroded lesions showed cleavage with haemorrhage at the junction of the superficial and reticular dermis.

At post-mortem there was amyloid infiltration of the endocardium between the myofibres and strikingly in the walls of the intramural branches of the coronary arteries, producing virtual

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occlusion. Amyloid infiltration was present in the pulmonary vessels, colon, tongue, peripheral nerves and vessels of the liver, spleen and adrenal glands. The sinusoids of the liver and spleen and the adrenal parenchyma were well preserved. In the kidneys the glomeruli were spared but mild amyloid infiltration was present in the vessels and parenchyma. Pronounced amyloid infiltration of the vessels of the bladder submucosa was found. The bone marrow was of normal cellularity and there was no excess of plasma cells. No sites of chronic inflammation or tumour were present.

DISCUSSION

The post-traumatic occurrence of haemorrhagic bullae on the objectively abnormal and normal skin of cutaneous amyloid as seen in this case conforms with previous reports of intra-lesional haemorrhage (Hurley & Weinberg, 1964), epidermolysis bullosa acquisita (Muller, Sams & Dobson, 1969) and bullae of the skin and mucous membranes (Northover *et al.*, 1972). Similarly, the ease with which raised central venous pressure may produce peri-orbital haemorrhage has been noted under the title of 'post-proctoscopic purpura' (quoted by Glenner, 1980). In our case the Valsalva manoeuvre was artificially supplied by an Ambu bag as opposed to the abrupt checking of the respiratory pattern accompanying dilatation of the anal sphincter.

The patient's death illustrates that in 'primary systemic amyloidosis' radiographic changes may be minimal but that the heart failure can be progressive and unresponsive to treatment. Arrhythmias, sometimes induced by digitalis, are a common terminal event (Buja, Khoi & Roberts, 1970). Our attribution of the patient's haematuria to amyloid infiltration of the vessels of the bladder duplicates a case described by Chew *et al.* (1975).

It was impossible to investigate the case immunologically. Tentatively the patient's B-fibrillosis (Glenner, 1980) was attributed to acquired systemic monoclonal protein amyloidosis.

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Fatal chronic cutaneous herpes simplex associated with thymoma and hypogammaglobulinaemia

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SUMMARY

A patient is described who suffered from cutaneous herpes simplex for 9 years. A mediastinal tumour had been demonstrated radiologically 10 years prior to the onset of the skin disease and at autopsy the tumour was identified as a spindle cell thymoma. The patient also had hypogammaglobulinaemia.

The association of thymoma with hypogammaglobulinaemia is well documented (Asherson *et al.*, 1979). Less often there is an association with skin diseases such as pemphigus (Stillman & Baer, 1972), scleroderma and systemic lupus erythematosus (Takagawa & Hayakawa, 1974). Chronic cutaneous herpes simplex has been reported in patients with impaired immunological competence (Logan Tindall & Elson, 1971) but its occurrence in patients with a thymoma is exceedingly rare. The only other case appears to be that described by Takagawa & Hayakawa (1974). In their patient, cutaneous herpes simplex had been present for 5 years and was also associated with systemic lupus erythematosus and red cell aplasia.

CASE REPORT

A 61-year-old Jamaican presented in 1961 with a chest infection. This responded rapidly to antibiotic therapy but a chest X-ray revealed an abnormal shadow in the anterior mediastinum. The lesion was considered to be a pericardial cyst. No change in size was observed radiologically over the next 5 years and the patient was discharged.

In 1979, she was referred to the dermatology department with a rash over her lower back, buttocks, upper thighs and genitalia (Fig 1.). The rash had been present in that distribution for at least 10 years and in 1970 the patient had been seen by a general physician for the same condition. However, the patient had been lost to follow-up and no record could be found of the diagnosis at that time. Examination showed an extensive vesicular rash in the distribution already described, with ulceration, scarring and post-inflammatory hyperpigmentation. Investigations revealed a mild iron deficiency anaemia (Hb 12 g/dl; serum iron 5 μmol/l and TIBC 39 μg/l) with a normal white cell and differential count. Total plasma proteins were reduced (42 g/l) with a low globulin fraction (15 g/l). Electrophoresis

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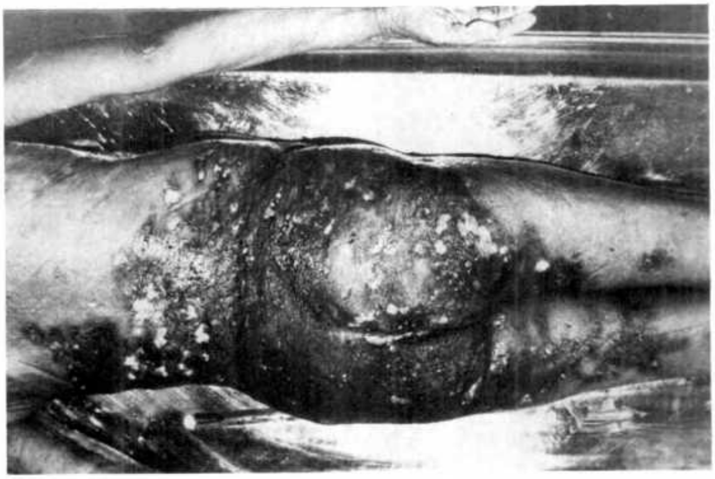

FIGURE 1. Cutaneous herpes simplex.

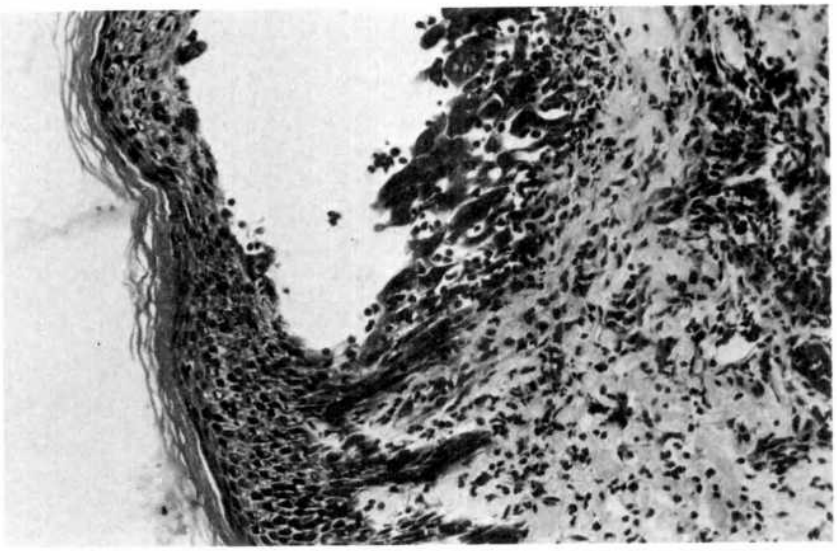

FIGURE 2. Skin biopsy showing an intraepidermal bulla with giant cell transformation in the cells at the edge (H & E, × 56).

confirmed severe hypogammaglobulinaemia with assay values of IgG 4·8 g/l, IgA 0·4 g/l and IgM less than 0·1 g/l. Other tests of liver function (prothrombin time, transaminases and alkaline phosphatase) were normal. Chest X-ray showed that the mediastinal shadow had not changed in appearance since 1965. Swabs taken from the skin ulcers were sterile and unfortunately specimens for viral studies were lost during transport. A skin biopsy (Fig. 2) showed complete ulceration of the epidermis with a non-specific inflammatory reaction. The clinical diagnosis was considered to be that of pemphigus associated with thymoma and the patient was started on prednisolone 10 mg three times daily. However, she died 4 days later in respiratory failure before detailed tests of cell mediated immunity could be performed.

Autopsy confirmed that the mediastinal tumour (110 g) was a benign thymoma of spindle cell type. Extensive sampling of the skin showed intraepidermal bullae, giant cell transformation and ballooning degeneration of the epidermal cells. Viral inclusion bodies were not seen. The dermis showed a severe mixed inflammatory cell reaction. Electron microscopy of the keratinocytes (Fig. 3) showed numerous 130 nm viral particles consistent with herpes simplex hominis. The lungs displayed herpes simplex pneumonia. Direct immunofluoresence studies on the skin for immunoglobulin and complement were negative.

Blood was not retained to enable serotyping of the virus. In the light of the autopsy findings a careful search was undertaken of the 1960 histopathology reports and the original skin biopsy was

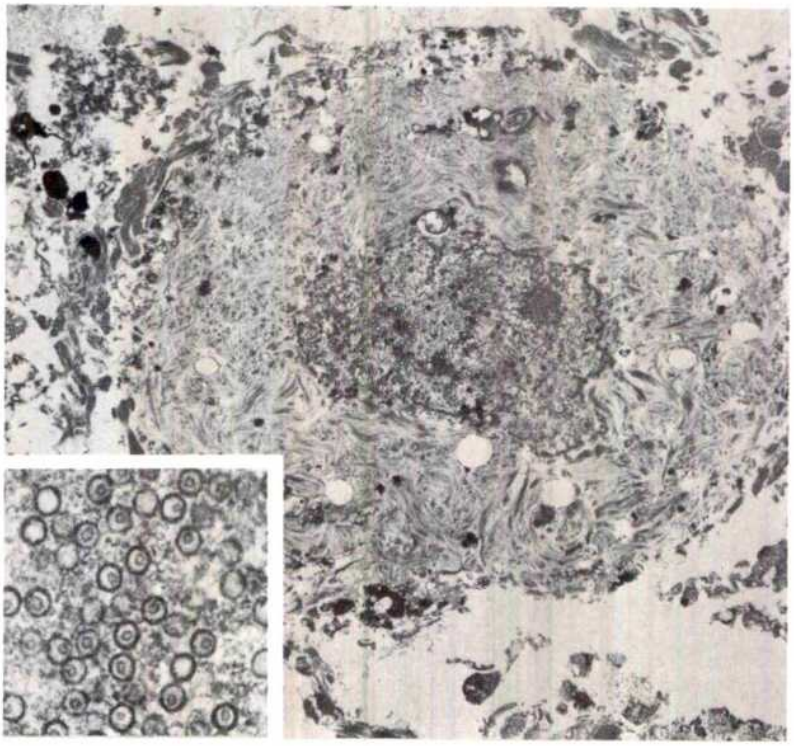

FIGURE 3. Electron micrograph of epidermal keratinocytes showing numerous intranuclear virus particles (×5250). Inset shows virions with the morphological features of herpes simplex hominis (×35,000).

retrieved. It showed changes similar to the autopsy biopsy and electron microscopy on paraffiin embedded material confirmed the presence of virus particles. In 1960, it had been reported as herpes simplex!

DISCUSSION

Infection with herpes simplex virus (HSV) is extremely common and in most instances is a short self-limiting disease. HSV may also occasionally present as a chronic cutaneous condition.

According to Logan *et al.* (1971), chronic cutaneous herpes simplex only occurs in individuals with altered immunity and this view is supported by the presence of hypogammaglobulinaemia in our patient. The exact factors which limit HSV infection are still uncertain but herpes simplex has been described in patients with both antibody deficiencies and abnormalities in cell-mediated immunity (Logan *et al.*, 1971).

The cause of hypogammaglobulinaemia in our patient remains uncertain as death occurred before detailed immunological tests could be performed. However, increased numbers of T suppressor lymphocytes with an associated reduction of B cells in the peripheral blood has been described in patients with thymoma (Asherson *et al.*, 1979). As in this case, thymomas associated with hypogammaglobulinaemia invariably contain epithelial elements. The thymoma present in the case reported by Takagawa & Hayakawa (1974) was also of spindle cell variety.

Montgomerie, Becrof & Croxson (1969) showed that, in renal transplant patients receiving immunosuppressive therapy, cutaneous herpes simplex carried a significant potential for progression into a disseminated form. It is therefore of interest that our patient died from herpes simplex pneumonia, shortly after commencing treatment with prednisolone. As stated by Logan *et al.*, (1971), it is because of this serious potential, that the early diagnosis of chronic cutaneous simplex assumes paramount importance.

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We are grateful to Dr R. Church for kind permission to report this case.

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Brief Communication

Herpes simplex virus and eczematous skin

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Husain & Somerville (1964) reported the presence of herpes simplex antigen in the exudate obtained from the eczematous skin of sixteen of forty-four patients (36%) thus raising the question of a viral flora in abnormal but clinically uninfected skin. Later Leyden & Baker (1979) isolated herpes simplex virus from eczematous lesions with discrete vesicles, but failed to recover virus from acute lesions which were neither vesicular nor ulcerative. We too have studied non-vesicular eczematous skin and failed to demonstrate the presence of herpes simplex virus.

Patients, aged between 5 and 72 years, admitted to St John's Hospital for Diseases of the Skin for treatment of their atopic eczema, were studied within 3 days of admission. Samples were taken from one or more eczematous lesions by scraping dry material into a sterile bijou bottle with a sterile scalpel blade; similar scrapings were fixed to a microscope slide using acetone. A swab sample was taken from the scraped area of the lesion by rubbing a moistened cotton-tibbed swab vigorously across the lesion and breaking off the swab tip in virus transport medium containing 0·2% bovine albumin. The swabs were then transported to the laboratory in wet ice, and inoculated into cell culture the same day.

The dry scrapings were ground-up in distilled water and the debris examined in the electron microscope. Virus isolation from the swabs was attempted on cultures of human embryo lung fibroblasts maintained at 37°C for 3 weeks. The fixed smears were kept at −30°C until examined for virus antigen by an indirect immunofluorescence test using a hyperimmune rabbit anti-herpes simplex serum (Division of Microbiological Reagents & Quality Control) and FITC-conjugated sheep anti-rabbit immunoglobulin serum (Wellcome Reagents Ltd). All smears were satisfactory with many cells present.

A pilot study on fifteen patients with eczema and eight patients with psoriasis using culture methods alone, failed to reveal any virus. A more comprehensive study of forty-seven lesions from twenty-five patients with atopic eczema also failed to demonstrate herpes simplex virus (Table 1). One electron microscope sample revealed poxvirus particles resembling molluscum contagiosum and it is of interest that only in this patient, a 10-year-old girl, were the lesions described as clinically infected. *Staphylococcus aureus* resistant to penicillin was also grown from this patient's lesions but only in small numbers.

In common with Leyden & Baker (1979), we have failed to demonstrate herpes simplex virus in lesions of atopic eczema which were not vesicular and were not clinically described as 'infected' although one patient yielded pox virus group particles from 'infected' eczema lesions in the absence of significant numbers of bacterial pathogens. These results are in contrast to the earlier report of Husain & Sommerville (1964). In the present series virus was sought by three separate techniques: virus isolation, electron microscopy and immunofluorescence microscopy. Although herpes simplex virus may fail to grow if not kept sufficiently cool in the interval between sampling and inoculation into cell culture, this should not affect the microscopy results. Leyden & Baker (1979) suggested that the immunofluorescent antibody technique used by Husain and Sommerville may not have been

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TABLE 1. Samples from patients with atopic eczema

Number of patients	Number of lesions sampled	Number of specimens examined		
		Cell culture	Electron microscopy	Immunofluorescence
25	47	47	46	28*

* Only one lesion tested from twenty-two patients.

specific and might therefore reflect false positive fluorescence from some other material present in the lesions. However it is also possible that the patients studied by Husain & Sommerville (1964) may have had the previously unrecognized clinical presentation described by Leyden & Baker (1979) with less obvious superficial vesicles, which they originally thought were only excoriations. We, on the other hand, have found no evidence of the presence of herpes simplex virus in patients with an acute exacerbation of atopic eczema.

ACKNOWLEDGMENT

We are grateful to the Consultant Staff of St John's Hospital for permission to sample patients under their care.

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British Journal of Dermatology (1981) **105**, 477–482.

Comment

UV-A and the skin

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Ultraviolet-A radiation (UV-A) is defined in wavelength terms as that part of the ultraviolet spectrum lying between 320 and 400 nm. The solar spectrum at the earth's surface includes UV-A, the solar UV-A irradiance being considerably greater than that of UV-B (290–320 nm). Interest in the actions of UV-A has recently been heightened by the discovery that in the presence of the furocoumarin, 8-methoxypsoralen, UV-A can be used with beneficial effect in the treatment of chronic plaque psoriasis (Parrish *et al.*, 1974a) and some other cutaneous disorders. The response to treatment appears to depend upon the ability of 8-methoxypsoralen to form mono-adducts and cross-links with DNA after excitation by UV-A, leading in turn to a reduction of DNA synthesis and cell division.

Although the phototoxic action of UV-A combined with psoralen in skin has been the subject of intense study over the past six years, the fact that UV-A has important biological effects on its own has received comparatively little attention. The subject deserves critical examination because of widespread concern about the proliferation of private health centres offering UV-A irradiation in solaria or sunbeds, thereby purporting to benefit the health of the purchasers of these facilities.

Establishment of the photobiology of UV-A requires the use of UV sources in which the emission is almost entirely confined to the UV-A wavelength range. Because of the much greater sensitivity of most biological systems to UV-B and UV-C, even minor 'contamination' of the emission spectrum by shorter wavelengths could prejudice the validity of observations on responses to UV-A. A number of studies reported in the literature suffer from this defect, and will not be mentioned here. UV-A radiation is lethal to a number of species of micro-organisms including *E. Coli* (Webb, 1977) but whether DNA or some other target molecule is involved is not at present clear. Mutants have been produced in cultures of *E. Coli* by irradiation with UV-A (Kubitschek, 1967). Little is known about the effect of UV-A on mammalian cells, although UV-A is known to be lethal to Chinese hamster cells (Danpure & Tyrrell, 1976).

THE ACTIONS OF UV-A IN MAN

Approximately 50% of incident UV-A radiation reaches the germinative component of human epidermis and about 35–50% reaches structures in the upper dermis (Everett *et al.*, 1966). UV-A is at least two orders of magnitude less effective in producing erythema in human skin

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than UV-B, the minimal erythemal dose (MED) being about 20–30 J/cm^2 (Parrish *et al.*, 1974b; Parrish *et al.*, 1976). The time course of the erythemal response to UV-A depends on the dose and on the wavelengths within the UV-A range that are involved. Kaidbey & Kligman (1979) used filtered and reflected UV-A from a 150 W Xenon arc to demonstrate that, with doses of 50 J/cm^2, redness appeared immediately after exposure without latency and lasted for 24 h or more. With threshold doses the erythema was much more short-lived. There was no identifiable biphasic response. It is, however, difficult to rule out the possibility that thermal effects might play a part in the immediate phase. In addition to direct erythemogenic activity, UV-A may augment the action of subsequently administered UV-B (Willis, Kligman & Epstein, 1973). It also causes a diminution of epidermal DNA synthesis 24 h after irradiation followed by a marked increase at 48 h (Willis *et al.*, 1973). Epidermal histological changes are inconspicuous in UV-A irradiated skin. UV-A irradiation causes both immediate and delayed melanin pigmentation (Pathak *et al.*, 1962). The energy required for delayed hyperpigmentation due to UV-A is much greater than that for UV-B and is not dependent on production of visible inflammatory changes.

Whether long-continued irradiation of skin by UV-A is by itself capable of evoking skin cancer is uncertain. This is partly because of technical difficulties. Most available sources emit 'stray' medium or short wavelength ultraviolet which may be biologically important in chronic irradiation experiments. The advent of high delivery rate laser sources has not overcome this problem completely because of local thermal effects. Although present available evidence suggests that under ordinary conditions UV-A is not carcinogenic, the matter must remain *sub judice* until more precise evidence is available (Ultraviolet Radiation. Environmental Health Criteria 14. W.H.O. Geneva, 1979, pp. 57–58).

The related possibility that UV-A may enhance the carcinogenic action of UV-B has been examined by Forbes, Davies & Urbach (1977). No evidence of potentiation has emerged. However, the possibility that chronic continuous high dosage UV-A with or without concomitant UV-B causes an increased incidence of skin cancer must remain until further research has been done. There is also no evidence to implicate UV-A in the production of degenerative changes in dermal connective tissues (solar elastosis) following chronic sun exposure; indeed the action spectrum for production of this change is unknown.

The lens of the eye is rich in tryptophan, which has marked UV-A absorbing properties. There is abundant animal and some human evidence to show that chronic exposure to wavelengths of UV of 320 nm and below causes severe toxicity in the cornea and lens of the eye. Evidence that UV-A causes eye damage is, however, incomplete. Bachem (1956) showed that cataract formation in the rabbit and guinea-pig occurred readily in response to wavelengths up to 313 nm. Prolonged exposure to 334–365 nm, involving total cumulative dosage of 3000–5000 J/cm^2 also caused lens opacities in these species. Long wavelength ultraviolet causes corneal damage. Pitts & Cullen (1978) irradiated the rabbit eye with 300–400 nm ultraviolet and detected corneal damage after administration of 50 J/cm^2 or more at 345 nm. No lens damage occurred following dosage of up to 162 J/cm^2. Recent work using ultraviolet lasers has contributed little to this important problem. Lenticular cataracts resulting from high irradiances over a short time period could be due to generation of local heat—a well recognized cause of lens opacities. In summary, present animal evidence suggests that prolonged UV-A exposure of the same irradiance as that occurring in natural sunlight may cause corneal and lens damage. Interactions between medium and long wavelength UV which could be of great practical importance in this context do not appear to have been studied. Retinal damage is unlikely, since the cornea and lens are highly effective absorbers of UV-A.

DOES UV-A RADIATION HAVE ANY BENEFICIAL EFFECTS?

UV-A radiation promotes a feeling of well-being in some individuals. This may be due to the psychological effect of its tanning action. The only recognized beneficial physical effect of ultraviolet radiation is the promotion of vitamin D synthesis, the action spectrum for which lies within the UV-B range. Repeated UV-A exposure (without 8-methoxypsoralen) led to improvement of psoriasis in a small group of patients (Parrish, 1977) although the cumulative dosages were high (1,800–6,980 J/cm^2) involving 13–32 treatments, and clearance was incomplete in some patients, with a marked tendency for relapse. van Weelden, Young & van der Leun (1980) compared the response of psoriasis to UV-B alone, with combined UV-B and UV-A. They found no evidence that addition of UV-A enhanced the therapeutic response to UV-B under the conditions of their study. Before dismissing claims for beneficial physical actions of UV-A it must be recognized that investigations of its physiological actions are extremely incomplete. For example, the effects of UV-A on immune function, blood chemistry, cutaneous neurophysiology and endocrinological processes in the skin have received little or no attention.

UV-A LAMPS AND SUNBEDS

The subject of risks and benefits in relation to UV-A radiation has become of considerable importance as a consequence of the proliferation of private 'clinics' and beauty salons for the purpose of administering UV-A on a regular basis, usually for cosmetic reasons, to members of the public. There is also considerable concern about the marketing of sunbeds for use at home for the same purpose. These facilities have been widely advertised in the lay press. Some UV-A solaria consist of high pressure mercury vapour lamps with filters to reduce or eliminate shorter wavelength UV and this may also incorporate unfiltered UV-B sources as 'boosters'. However, the average sunbed consists of a transparent perspex plate for the patient to lie on, under which is located a bank of UV-emitting fluorescent low pressure mercury discharge tubes. Approximately 80% of the radiant energy output of these lamps is in the region of 315–400 nm, while 0·5% is below 315 nm (Diffey, Challoner & Key, 1980). There is no UV-C, and the remainder of the energy is emitted in the visible and infrared region. The spectral output is modified by the perspex plate interposed between the tubes and skin which attentuates the shorter wavelengths. Skin is exposed, front and back, for about 30 min per day. With such a unit the skin might receive 20 J/cm^2 of UV-A which approximates to twice the dose which it would receive if it were exposed for a similar length of time to the mid-day sun in the tropics. Several important factors have to be taken into account in assessing risk to the public in the unsupervised use of these sources. To the amount of UV-A received from the sunbed must be added the UV-A which would ordinarily be received from daily sunlight, in order to arrive at the total radiant exposure to UV-A. UV-A may also enhance the biological effects of UV-B—both from sunlight and from the sunbed itself. Finally, unlike UV-B, UV-A only causes erythema in very high dosage and there is thus little to discourage the over-enthusiastic user. The possibility that a severe phototoxic eruption may occur in patients receiving photosensitizing drugs including sulphonamides, nalidixic acid and wide spectrum antibiotics is the major short term risk in users of sunbeds and other UV-A lamps. A similar acute reaction can also occur in patients applying cosmetics containing photosensitizers including the furocoumarins. The tanning effect of UV-A offers little protection against the burning action of UV-B and thus users, lulled into a false sense of security by a cosmetically impressive tan, may suffer severe sunburn (Kaidbey & Kligman, 1978). On present evidence the major long term fear must be the insidious development of cataracts and it is especially alarming that some vendors claim in their advertising copy that UV-A is 'harmless to the eye', so that users of their lamps could dispense with any form of eye protection. There is as yet no experimental evidence to support the view that UV-A is carcinogenic in skin. Nevertheless, until more careful studies have been done, this possibility cannot be discounted.

Despite its limitations and incompleteness our currently available information argues in favour of a cautious approach to the use of UV-A especially for cosmetic purposes. The use of UV-A sunbeds and the like should be confined to centres in which irradiation is carried out under supervision by qualified individuals, possibly physiotherapists. Eye protection should be invariably worn, and users should be made aware of the possible dangers of subsequent sunlight exposure. Particular care should be taken to avoid the use of psoralen-containing sun tanning lotions prior to UV-A exposure. Customers receiving concurrent medical treatment should check that the tablets they are receiving do not cause photosensitization. Advertising copy and users' manuals should clearly state the potential hazards of the lamps, and the precautions necessary to avoid them. The U.K. Health and Safety Executive and the U.K. National Radiological Protection Board (1977) have adopted threshold limits for occupational exposure to UV-A radiation. It is disturbing that UV-A irradiation is apparently subject to no restriction whatever in sunbed centres and in the homes of domestic users.

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British Journal of Dermatology (1981) **105**, 483–485.

Correspondence

Treatment of acute febrile neutrophilic dermatosis (Sweet's syndrome) with colchicine

SIR, We recently saw a patient with Sweet's syndrome, who showed a very rapid improvement with colchicine.

The patient was a 29-year-old man with a 5-day history of dusky-red, tender, swollen plaques on the left forehead and the right upper eyelid associated with aphthous stomatitis. Five months previously he had suffered from similar tender red plaques on the face with severe arthralgia involving the hip and knee joints, oral aphthae and neutrophilic leukocytosis. The diagnosis of Sweet's disease was made on the basis of the clinical and histopathological features of the skin lesions. At that time he did not respond to oral potassium iodide 0·5 mg three times daily for 5 days (Horio *et al.*, 1980) but he gradually improved with oral prednisone 10 mg daily for 4 weeks. On this occasion he was treated with 0·5 mg of oral colchicine three times daily. Within 2 days the tender erythematous plaques and aphthae began to recede and they disappeared within 1 week without the development of arthralgia.

Since Sweet's syndrome shows massive polymorphonuclear leukocyte (PMN) infiltration in the dermis with increased chemotactic activity of the peripheral blood PMNs in the acute stage (Oseroff *et al.*, 1981), we surmise that colchicine acted as it does in Behçet's disease and other diseases in which PMNs play a crucial role (Matsumura & Mizushima, 1975; Hanzen & Michel, 1979). Colchicine is known to inhibit the chemotaxis of PMNs and it decreases leukocyte motility *in vitro*, blocks leukocyte adhesiveness, and stabilizes lysosomal membranes. In our case colchicine seemed to improve the signs and symptoms of Sweet's syndrome and appeared to be more effective than other drugs we had used. We suggest that further trials of this drug for Sweet's syndrome should be performed.

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Mycosis fungoides responding to tetracycline and griseofulvin

SIR, The article by Shelley (1981) concerning two cases of mycosis fungoides (MF) clearing after treatment with demethylchlortetracycline and griseofulvin, respectively, prompts me to report similar observations made in two patients a few years ago, but not published because the observed effect of treatment could not be repeated in other patients.

Case 1

In 1969 a 43-year-old female was referred in the tumour stage of MF. The skin disease began 20 years previously and tumours had appeared in 1965. The diagnosis of MF was confirmed histologically but extracutaneous dissemination could not be demonstrated at that time.

Because of tumour necrosis and signs of cellulitis, tetracycline 1 g daily was instituted, and the tumours that had been growing steadily now ceased to grow and during the next 2 weeks regressed. After 3 weeks of treatment the tumours were completely gone while MF-plaques on the trunk remained unaltered. After 2 months, however, and despite continuous treatment, new tumours reappeared on the arms and thighs. Bleomycin was now started with a total dose of 300 mg given, and this treatment resulted in partial remission of the tumours.

Six months later tetracycline 1 g daily was repeated and again the ring-shaped tumours regressed. Now, however, the patient showed signs of cerebral involvement, and she was referred to another department where she ultimately died.

Case 2

In 1972 a 69-year-old man was referred in the tumour stage of MF, histologically confirmed. The disease had lasted for 2 years and tumours had been present on the scalp, trunk and lower extremities for 6 months.

The general practitioner had for several months been able to control the pruritus and the skin lesions with griseofulvin 500 mg daily. This unorthodox treatment was prescribed because of the suggestive name of the disease.

Naturally, the hospital staff could not accept this non-scientific approach and I forgot this observation until the article by Shelley appeared. Later, tetracycline was tried on a further four or five cases of MF but without the slightest effect and therefore this observation also was forgotten.

The observations of Shelley are extremely important and further studies are needed to confirm them and to find out the working mechanism of antibiotics in lymphoproliferative diseases such as MF.

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REFERENCE

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Halogenation and topical steroids

SIR, The paper by Allenby & Sparkes (1981) raises some interesting points. It is now generally accepted that the potency of a topical steroid, either in terms of clinical activity or side effects, is not dependent on the degree of halogenation but may well be related to the identity of the substitution at the 11 position, e.g. a β-OH group in the case of clobetasol and a keto-group in clobetasone. The fact that the more potent steroids tend to be halogenated may be a red herring. However, some doubt may be cast on this view when one notes that clobetasone butyrate, a halogenated compound classified by MIMS as 'moderately potent' is presented at a concentration of 0·05% whereas the 'potent' steroids, betamethasone valerate (halogenated) and hydrocortisone 17-butyrate (non-halogenated) contain a 0·1% of active ingredient.

The use of occlusive dressings to alter the clinical activity of topically active steroids is an old concept and Munro (1976) showed that 60 g of hydrocortisone 17-butyrate 0·1% applied daily under occlusion caused a similar degree of HPA axis suppression to 30 g of betamethasone 0·1% applied daily. Marten *et al.* (1980) could, however, detect no significant difference between the suppression produced by hydrocortisone 1% or hydrocortisone 17-butyrate 0·1% when applied without occlusion in amounts of 30 g or 60 g to children suffering from eczema.

The authors rightly suggest that clinical trials can often lead to erroneous results if the 'wrong' model is chosen. This is true when treatments of eczema are shown to exert equal clinical activity. It is also a principle of good clinical research to choose end points which are as objective as possible and to measure the findings over a reasonable treatment period. To assess the effect of topical steroids in psoriasis, a disease not known for being highly steroid-responsive, on the single subjective criterion of global opinion over a short period, is to lay the study open to considerable scepticism.

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Zinc sulphate and alopecia totalis

SIR, We agree with Dr Ead (*British Journal of Dermatology*, **104**, 483) that oral zinc sulphate causes no improvement in alopecia areata after only 3 months treatment, but it does cause regrowth if the treatment is continued for a much longer period. We have treated 116 patients with alopecia totalis with Solvezink® 0·2 g three to six times daily for several years. No regrowth of hair was seen after 4–8 months of treatment, but within 2 or 3 years, thirty-five patients had full regrowth and forty-one patients had partial regrowth. We do not believe that this improvement in seventy-six of 116 patients was due to chance, since spontaneous regrowth is rare in alopecia totalis. Moreover if the drug was withdrawn for 2–3 weeks the hair began to fall out again, but it grew once more when the zinc was re-started.

There was no significant increase in plasma zinc during this treatment, and we cannot explain the mechanism of the action of the drug.

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British Journal of Dermatology (1981) **105**, 486–487.

Book Reviews

Recent Advances in Sexually Transmitted Diseases. 2. Edited by J.R.W.HARRIS (1980) Edinburgh: Churchill Livingstone. Pp 281. Price £18.00.

Before discussing the contents, praise is due to the publisher. This is a book which it is a pleasure to handle, to read and even to smell.

Dermatologists in training should be aware of current trends in venereology, as it used to be called, but older colleagues who might think it a minor speciality with be surprised to see how much is going on. There are more than twenty contributors and they avoid duplication. If the quality of the chapters varies, all are good and some outstanding.

Dr Willcox opens with a survey of international trends affecting control of sexually transmitted diseases, and this detailed survey of a wide field should be rewarding to any dermatologist.

Many of the chapters deal with clinical, laboratory and epidemiological aspects of urethritis in its various forms, and these will be of limited interest to British dermatologists, although the content was fascinating to the reviewer in showing how out of date his views were. Clinical syphilis rates only four pages, but more space is given to basic research into treponemal diseases and to diagnostic procedures, which are of interest to dermatologists. Treatment schedules are discussed, emphasizing the unsuitability of some antibiotics, and the difficulty in obtaining suitable formulations of penicillin.

Other chapters deal with subjects of mutual interest, such as warts, molluscum contagiosum and scabies. Most dermatologists should be familiar with advances in these fields, but they are well summarized, although with an emphasis which differs from ours. I am glad to note that the treatment of molluscum contagiosum 'does not really seem to give problems'!

Even though few dermatologists will read this from cover to cover, most should enjoy several chapters and be glad to refer to some of the less obviously appealing ones, such as chancroid or lymphogranuloma venereum, when in diagnostic difficulty. It is a pity that so many sexually transmitted diseases are still referred to ignorant dermatologists instead of to experts in genital medicine clinics. All the more reason for the former to buy this book, or to see that it is available in their departments.

D.SHARVILL

Clinical Trials. DANIEL SCHWARTZ, ROBERT FLAMANT and JOSEPH LELOUCH. Translated from French by M.R.J.HEALY (1980) London: Academic Press. Pp. 275, 16 statistical tables. Price £16.60.

The authors have made a contribution which is valuable for its discussion in depth of some of the features of planning a clinical trial, a subject about which there are many articles but few books. The formulation of the problem to be answered by a trial is examined in detail from two extreme view-points; firstly the 'explanatory' approach, designed for the advancement of scientific knowledge, and secondly the 'pragmatic' approach, which is intended to illuminate the practical choice between treatments under conditions similar to everyday use. Since any one trial will contain a blend of both approaches, compromises must be made. The authors urge that the necessary decisions should be made deliberately and by choice rather than by default, pointing out that if considered decisions are not made, the trial may be doomed to failure before any clinical work has been done.

The possibilities of coming to conclusions in error are examined in more detail than is often the case. There is much discussion of the need to study a sufficient number of patients and how to determine such a number. None of the recently proposed, and hence relatively untried, techniques for economising the number of patients required are mentioned.

Reasonably interesting examples have been selected, there is an index, the figures are clear, the type is agreeable and the binding seems secure. On the other hand, the language seems to have lost some of its sparkle in translation and at least one reference is missing from a fairly short list.

Although the discussion of clinical trial planning is not fully comprehensive, this book deserves a place on the departmental or library shelf, to aid in planning real trials rather than for students. However, I thought it rather expensive for a paperback.

M.F.CORBETT

British Journal of Dermatology (1981) **105**, 488–489.

News and Notices

British Society for Oral Medicine

At a meeting held in the Royal Dental Hospital, London, on 24 February 1981, it was decided to form a society named the British Society for Oral Medicine. The Society aims to promote the practice of oral medicine through teaching and research, and to encourage communication between those with an interest in any aspect of oral medicine.

The founder president is Professor Brian E.D.Cooke, and it is proposed to hold the next annual meeting in Cardiff in May 1982.

For further details contact: Dr M.M.Ferguson, Department of Oral Medicine, Dental Hospital, 378 Sauchiehall Street, Glasgow G2 3JZ.

E.S.D.R. Poster Awards

At the 11th Annual E.S.D.R. Meeting held at Nordwijkerhout in May 1981, the following Poster Awards were made:

(1) Friends of Bill Reed Committee ($800) 'Binding profiles of lectins to epidermal Langerhans cells'. G.Schuler, J.Linert, E.M.Shevach and G.Stingl, Innsbruck Medical School Austria, and N.I.H., Bethesda, MD, U.S.A.

(2) Italian Foundation for Research in Dermatology ($500) 'Phenotypic stability and genotypic alterations in a squamous cell carcinoma line from human skin'. P.Boukamp, W.Tilgen, D.Breitkreutz, R.Dzarlieva, R.Riehl and N.E.Fusening, German Cancer Research Centre and Department of Dermatology, Heidelberg, West Germany.

Fifth Worcester Dermatopathology Workshop

A 2-day Dermatopathology Workshop will be held on 22 and 23 July 1982 at the University of Massachusetts Medical School, Worcester, Massachusetts. The Workshop will include the study of 100 unknown histology slides of inflammatory dermatoses and skin tumours.

For further information contact: Jag Bhawan, M.D., UMass Medical School, Worcester, Massachusetts 01605 (U.S.A.): 617-856-2412.

John Milne Memorial Fund

Applications are invited for travelling scholarships funded by the John Milne Memorial Fund. These are for travel to dermatological meetings or to centres which the applicant wishes to visit to increase his/her knowledge and experience either of clinical problems or of research techniques. The scholarships are open to clinicians and scientists for travel during 1982 and the total value will not exceed £350.

Applications, including a full curriculum vitae and reasons for travel, should be sent to Professor R.M. MacKie, University of Glasgow, Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU, by 1 November 1981.

Zagazig Dermatology Conference, Egypt

The Second International Dermatology Conference will be held in Zagazig and Cairo from 30 January to 2

February 1982. The theme will be tropical dermatology. For details contact Mr William Chaveas, Executive Director, College of Physicians of Philadelphia, 19 South 22nd Street, Philadelphia PA 19103, U.S.A.

Advanced Course in Dermatological Photobiology

A course entitled 'Recent Advances in Dermatological Photobiology' will be held at the Institute of Dermatology, St John's Hospital for Diseases of the Skin, Lisle Street, London WC2, from 12 to 15 April 1982. This course is primarily designed for Senior Registrars and Consultants and will be chaired by Dr J. Hawk. Topics covered will include basic aspects of photobiology, instrumentation, phototoxicity, photoimmunology, the porphyrias, phototherapy and photoprotection.

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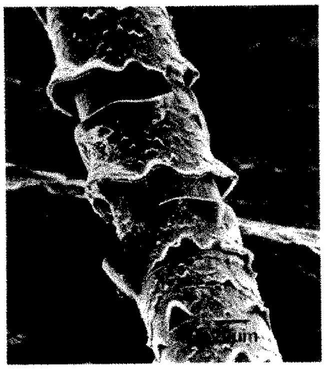

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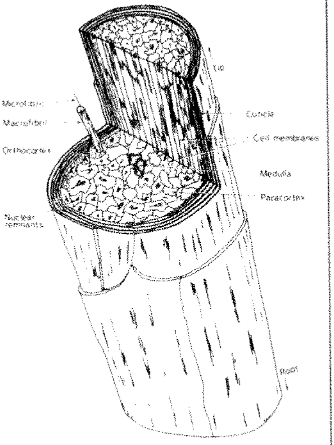

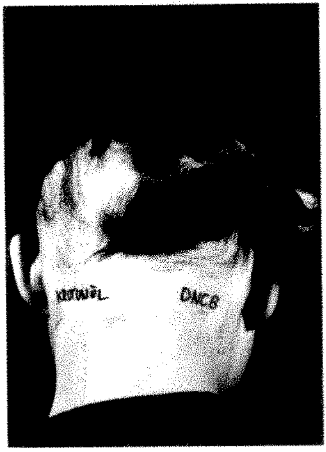

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(Proceedings of the International Dermatology Symposium (IDS), Berlin, October 13-15, 1980)

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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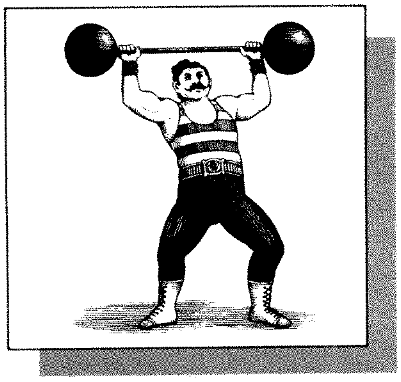

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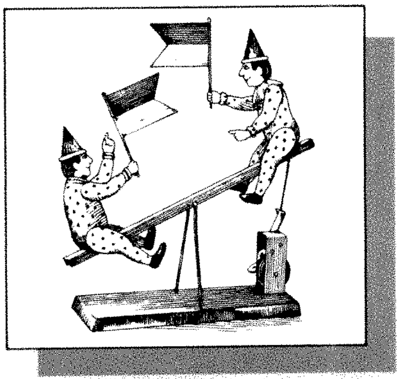
1. Intern Rapport, Medische Afdeling, Boehringer Ingelheim B.V.
2. Cahn, M.M. et al. Curr. Therap. Res. (1977) 2, 297.



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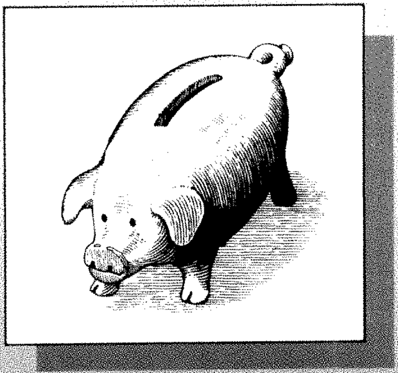
3. Turi, J.S. et al. J. Pharmac. Sci. (1979) 3, 275.



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Arthur Rook MA, MD, FRCP
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Addenbrookes Hospital, Cambridge

and **Rodney Dawber** MA, MB, ChB, FRCP
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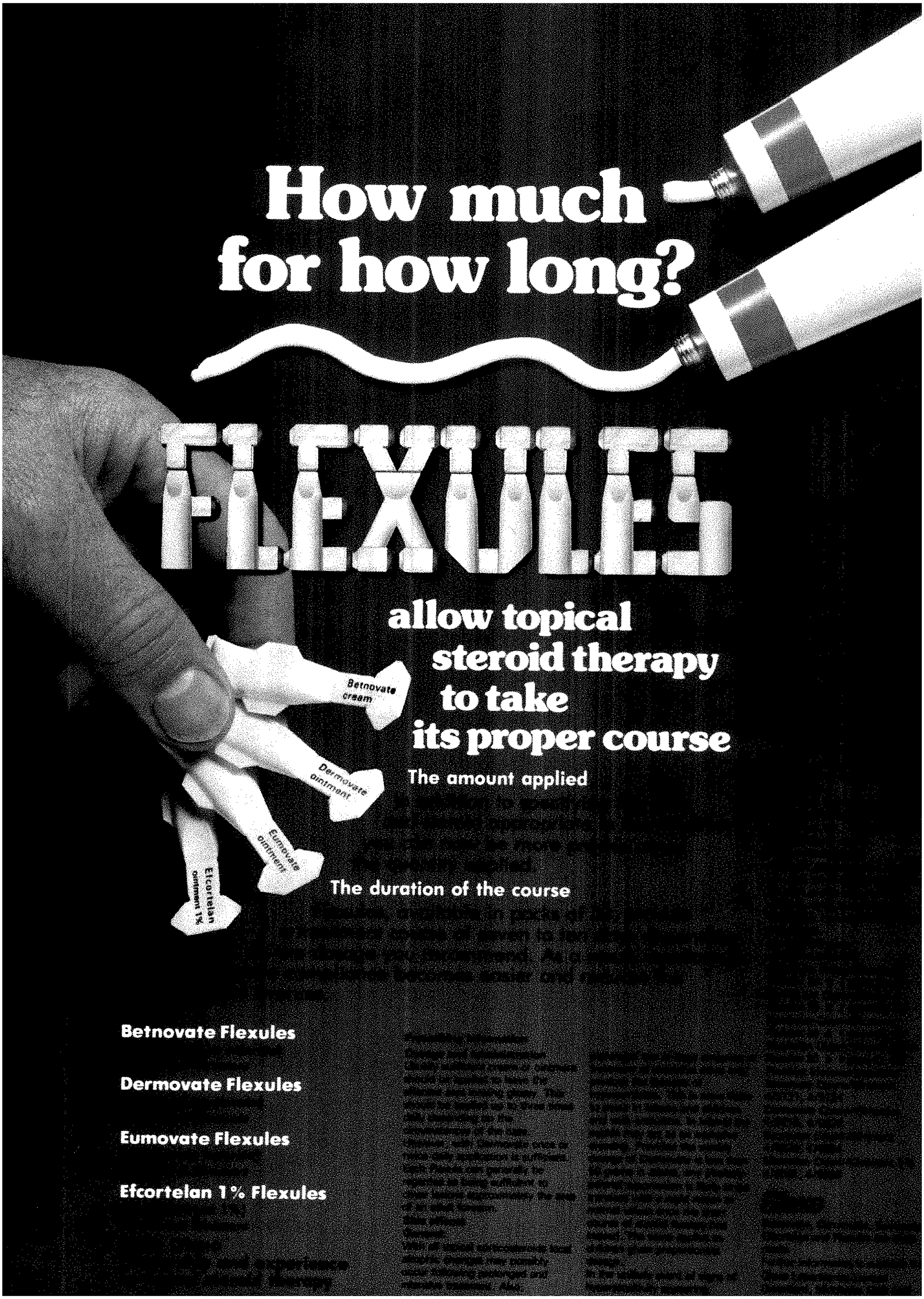

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The duration of the course

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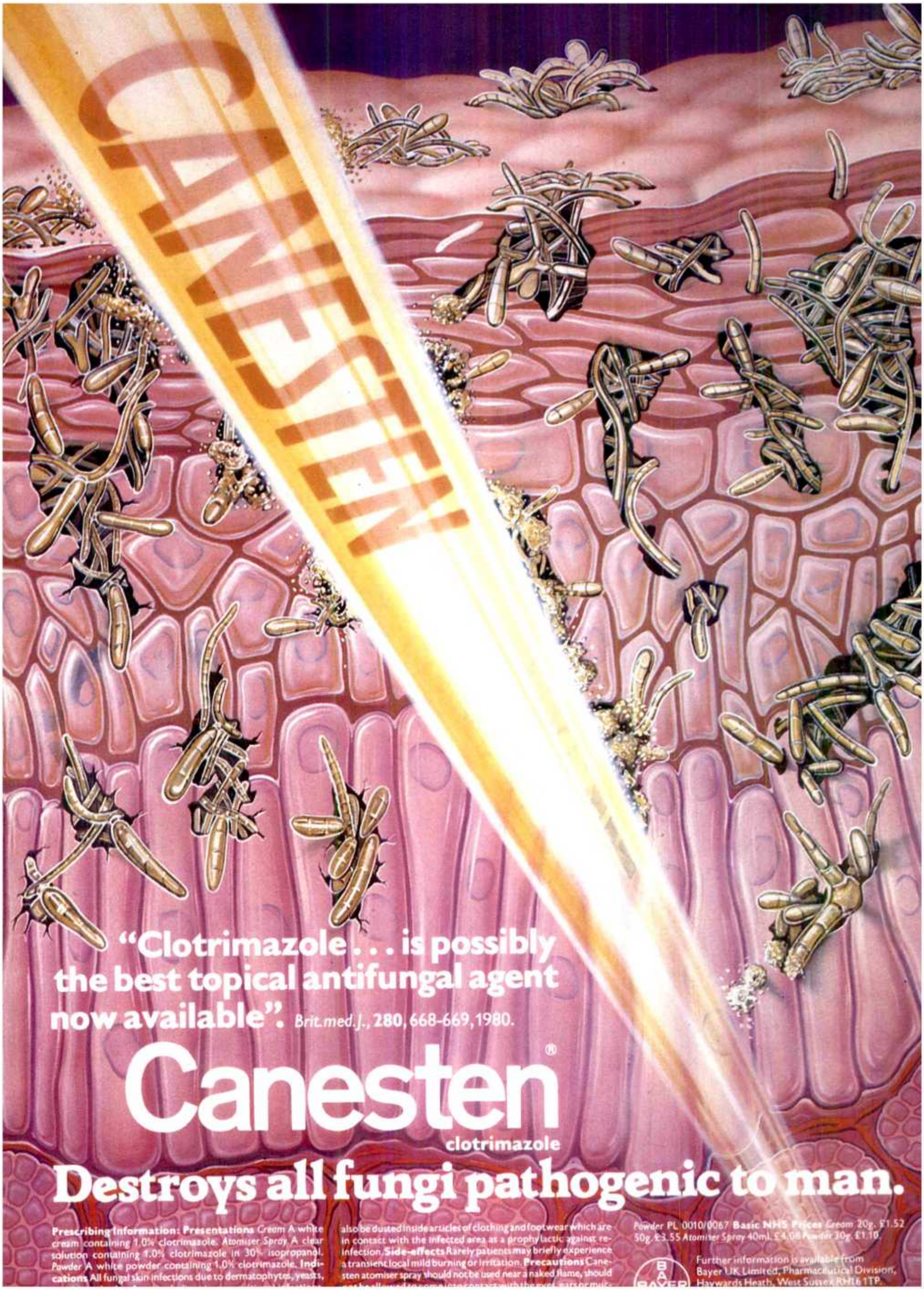
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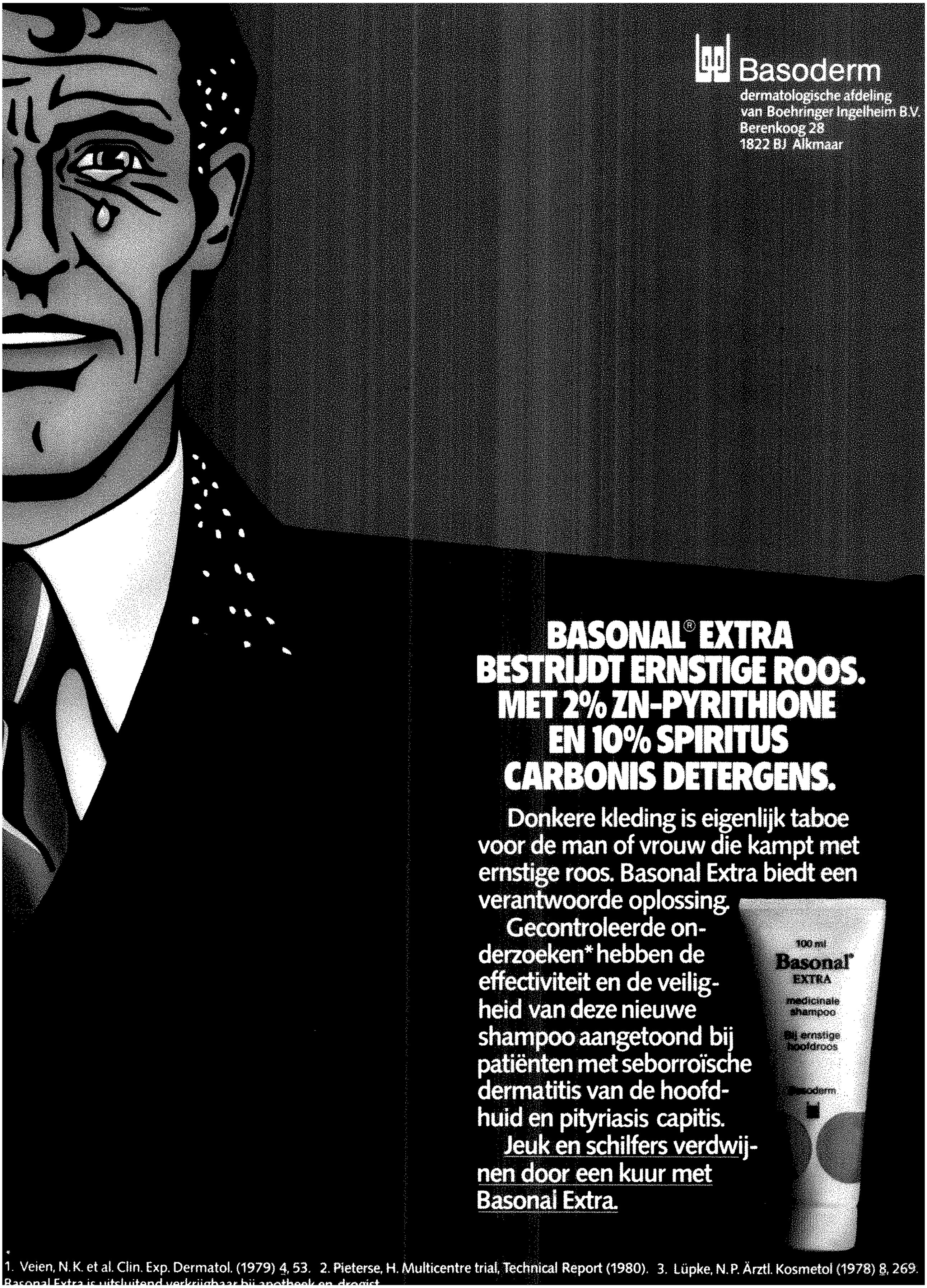

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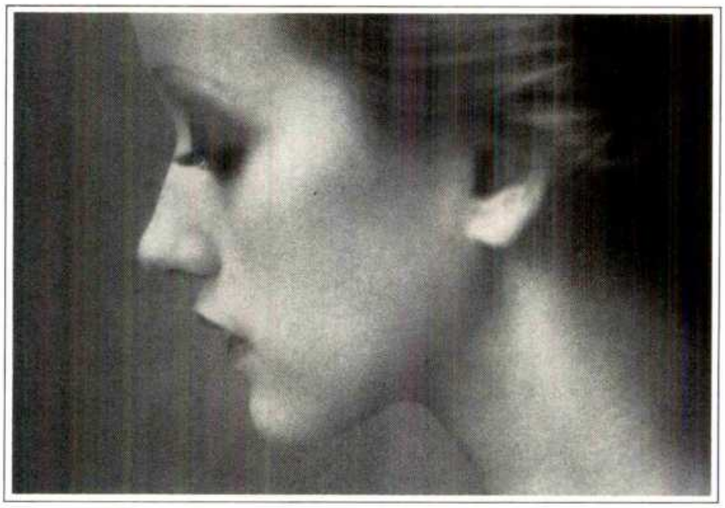

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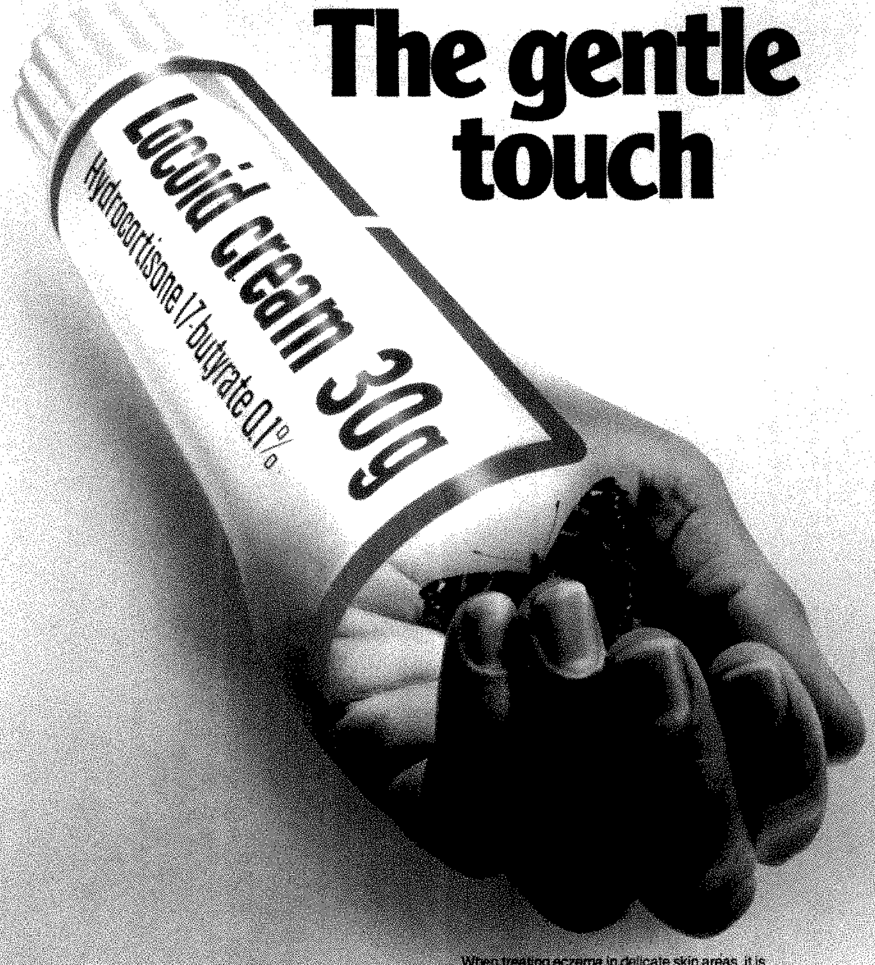

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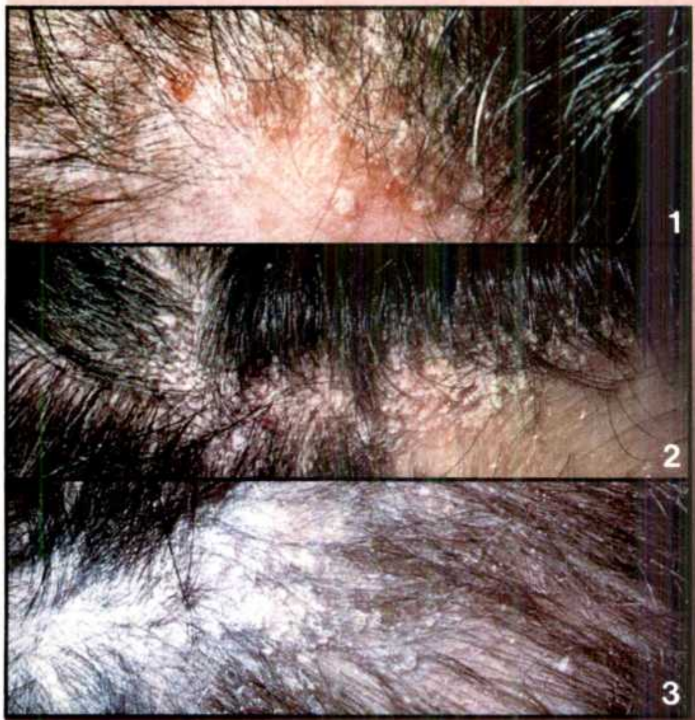

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Clinical and Laboratory Investigations

Unscheduled DNA synthesis in normal human skin after single and combined doses of UV-A, UV-B and UV-A with methoxsalen (PUVA)

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SUMMARY

The aim of this study was to measure unscheduled DNA synthesis (UDS) by autoradiography in normal human skin (1) after high dose UV-A, (2) after low dose UV-A applied before or after erythemogenic doses of UV-B, (3) after high dose PUVA and (4) after therapeutic doses of PUVA applied before and after erythemogenic doses of UV-B.

Single high dose UV-A exposure induced roughly 60% of the amount of UDS induced by equally erythemogenic doses of UV-B. Single low dose UV-A exposure did not induce UDS, nor did it significantly alter the amount of UV-B induced UDS when combined with UV-B exposure. Single high dose PUVA did not lead to UDS and had no influence on UV-B induced UDS when combined with UV-B exposure.

Our findings indicate: (1) erythemogenic doses of UV-A induce a considerable DNA excision repair; (2) low dose UV-A neither augments UV-B induced DNA repair nor does it inhibit the repair process; (3) no UDS was shown to occur after either high or therapeutic doses of PUVA. This was unexpected since psoralen-DNA monoadducts have been shown to be repairable by a mechanism similar to excision repair of pyrimidine dimers. It is therefore assumed that PUVA as performed for therapeutic purposes either preferentially induced interstrand crosslinks not repairable via the classical repair mechanism or the repair of monoadducts was below resolution in this study; (4) therapeutic PUVA doses apparently do not interfere with excision repair of UV-B induced DNA lesions.

Exposure of human skin to different UV waveband regions causes different dose related biologic

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effects. For a long time, most of the knowledge that had accumulated concerned the effects of UV-C (200–290 nm) and UV-B (290–320 nm), whereas UV-A (320–400 nm) had been thought to be more or less biologically inert. However, growing knowledge of UV-induced tissue damage and the availability of new potent radiation devices for research have led to increasing awareness of the fact that UV-A is photobiologically active in the skin (Parrish *et al.*, 1978). It causes erythema, pigmentation and light and electron microscopic changes if given in appropriate doses (Pathak, Riley & Fitzpatrick, 1962; Stern & Urbach, 1972; Parrish *et al.*, 1974, 1976; Willis & Cylus, 1977; Kumakiri, Hashimoto & Willis, 1977; Kaidbey & Kligman, 1979; Aberer *et al.*, 1980). Moreover, most photosensitivity reactions are mediated by UV-A. Combined exposures to UV-A and UV-B produce erythema and histological alterations in the skin which are characteristic for sunburn damage, even if both UV-A and UV-B are applied in suberythemogenic doses. Based on quantitative considerations this phenomenon has been termed photoaugmentation (Willis, Kligman & Epstein, 1973; Kaidbey & Kligman, 1975; Spiegel *et al.*, 1978) or photoaddition (Parrish *et al.*, 1974; Ying, Parrish & Pathak, 1974).

Erythema response, pigmentation, microscopic and ultrastructural events after exposure of human skin to different wave band regions of the UV spectrum and the psoralen-UV-A (PUVA) reaction are currently being investigated in detail. Studying the effects of equally erythemogenic doses of UV-A, UV-B, UV-C and PUVA, Rosario *et al.* (1979) found qualitatively similar histologic changes within the epidermis after all of these treatments, noting only quantitative differences and different time intervals between irradiation and the appearance of detectable alterations. The shorter wave bands affected the epidermis earlier and to a greater degree than the longer wave bands. By contrast, the longer wave bands produced more pronounced changes in the dermis than equi-erythemogenic doses of shorter wave lengths (Rosario *et al.*, 1979).

Controversial results have been reported on the effects of UV-A with and without psoralens on epidermal unscheduled DNA synthesis (Baden *et al.*, 1972; Willis *et al.*, 1973; Epstein & Fukuyama, 1975; Swanbeck *et al.*, 1977; Bishop, 1979; Bioulac *et al.*, 1980). This is partly due to the fact that in some of these earlier studies the UV-A doses employed were either ill-defined or too small to yield meaningful results. The present study was performed to determine the magnitude of excision repair of UV radiation-induced DNA damage, as reflected by unscheduled DNA synthesis (UDS) after equally erythemogenic doses of UV-A, UV-B and PUVA and of combined irradiation with UV-A and UV-B, and UV-B and PUVA.

MATERIAL AND METHODS

Subjects

For all experiments the previously non-sun-exposed buttock skin of eleven healthy Caucasian volunteers, with ages ranging from 20–35 years (skin types I and II) (Melski *et al.*, 1977; Wolff *et al.*, 1977), was used. Informed consent was obtained.

Radiation sources

UV-A. (a) 6000 W Xe arc source (Optical radiation Corp. V-4500) optically matched with a Jobin-Yvon HL 300 holographic grating monochromator. Half-band width 30 nm between 335 and 365 nm. Irradiance: $59{\cdot}0 \times 10^{-3}$W/cm^2 at 23 cm. (b) 2500 W Xe arc source (Schoeffel Instruments) plus doped liquid filter (7% $CuSO_4$ + 7% $CoSO_4$ in 0·1 M H_2SO_4) with a path length of 6·3 cm plus a Corning 7–60 (A) glass filter. Irradiance: $41{\cdot}0 \times 10^{-3}$W/cm^2.

UV-B:(a) 6000 W Xe arc source with a Jobin-Yvon HL 300 monochromator (as above). Half-band width 30 nm between 275 and 305 nm. Irradiance: $9{\cdot}0 \times 10^{-4}$W/cm^2. (b) Westinghouse fluorescent bulbs (FS 40) plus liquid filter (20% $NiSO_4$ + 8% $CoSO_4$ in H_2O) with a path length of 5·7 cm: 280–315 nm irradiance was $7{\cdot}0 \times 10^{-5}$W/cm^2.

Radiometry

All irradiance measurements were performed using calibrated cosine-corrected detectors at the same distance from the lamps as used during skin exposures. The irradiance produced by the UV-A sources was measured with an International Light, Inc., Model IL 442 radiometer which responds to 315–400 nm radiation with a peak response at 365 nm. The irradiance for the UV-B source was measured with an International Light, Inc., Model IL 700 radiometer system with a SEE 400 photodiode fitted with an interference filter with maximum transmission at 297 nm and with scattered light reduced by a factor of 10^{-5}.

Erythema grading

Erythema gradings were performed according to the definition given in Table 1 by two observers under identical lighting conditions.

TABLE 1. Erythema gradings

0	No erythema
+1	Pink erythema with definite borders (= MED or MPD response level)
+2	Red erythema but no oedema
+3	Intense erythema with oedema
+4	Fiery red erythema with oedema and blistering

Radiation exposures

The minimal erythema dose (MED) for UV-A and UV-B and the minimal phototoxicity dose (MPD) (Wolff *et al.*, 1977) for methoxsalen-UV-A were determined for each subject prior to the experiments. The MED is defined as the least UV-dose used elicting a +1 erythema reaction 24 h after exposure to UV-A and/or UV-B. The MPD is defined as the least UV-A dose used eliciting a +1 erythema reaction 72 h after exposure in an individual who has received 8-methoxypsoralen (0·6 mg/kg) 2 h prior to the UV-A exposure. The exposure doses used in the experiments were adjusted to the individual MED and/or MPD of each test subject with the aim of producing comparable erythema reactions with the various wavebands and PUVA. Despite the fact that the MED is not a physical dose measurement this approach was chosen because absorption of UV-energy by DNA depends on several factors (e.g. skin type, pigmentation, thickness of the horny layer) and thus varies considerably. A constant UV-dose would therefore not allow comparison between the biological effects in different individuals.

Skin areas measuring 5 × 5 cm^2 were exposed in each subject. In those experiments where two different UV wavebands were applied consecutively the first exposure was immediately followed by the second. The experimental design is detailed in Table 2. In experiments one and two we compared the effects of equally erythemogenic doses of UV-B and UV-A, and of UV-B and PUVA, respectively. Experiment three was performed to investigate the effects of exposures to low-dose UV-A before and after erythemogenic doses of UV-B. Experiment four delineates the effects of exposures to therapeutic doses of PUVA before and after erythemogenic

TABLE 2.

Experiment 1 (3 subjects)		Experiment 2 (2 subjects)	
Skin area 1	UV-B (2 × MED)	Skin area 1	PUVA (2 × MPD)
2	UV-A (2 × MED)	2	UV-B (2 × MED)
3	UV-B (3 × MED)	3	PUVA (2 × MPD)*
4	UV-A (3 × MED)	4	UV-B (2 × MED)*
5	Control (unexposed)	5	Control (unexposed)

Experiment 3 (3 subjects)		Experiment 4 (2 subjects)	
Skin area 1	UV-B (2 × MED)	Skin area 1	PUVA (1 MPD)
2	UV-A (1/4 MED), then UV-B (2 × MED)	2	PUVA (1 MPD), then UV-B (2 × MED)
3	UV-B (2 × MED), then UV-A (1/4 MED)	3	UV-B (2 × MED), then PUVA (1 MPD)
4	UV-A (1/4 MED)	4	UV-B (2 × MED)
5	Control (unexposed)	5	Control (unexposed)

* Biopsy after 24 h.

doses of UV-B. Table 3 shows the means and the ranges of the UV-doses required to obtain a +1 erythema with UV-A, UV-B and PUVA for all subjects.

Biopsies

Shave biopsies were performed under local anaesthesia immediately after irradiation. In experiments three and four, biopsy specimens from areas two and three were obtained at the end of the irradiation with the second light source; and from areas three and four of experiment two after 24 h (Table 2). Non-irradiated skin served as control.

Autoradiography

Immediately after biopsy the specimens were incubated at 37°C for 1 h floated in Medium 199 with ^{3}H-thymidine (20 μCi/ml; specific activity 5 Ci/mmol) and fixed in buffered neutral formalin. 5 μm sections were coated with Kodak nuclear track emulsion NTB-2, the exposure being 20 days at 4°C. The films were developed with Kodak D19 Developer and fixed in Fixon Ultrarapid (Daniel).

The number of sparse labelled cells (SLC) (3–15 grains per nucleus) per 1000 cells and the number of grains per SLC were counted for the entire viable epidermis. Three grains per nucleus were chosen as threshold count for defining sparse label since preliminary experiments

TABLE 3. Mean and range of MED and MPD values (11 subjects)

	UV-A (J/cm^2)	UV-B (mJ/cm^2)	PUVA (J/cm^2)
$\bar{x}$ ± s.d.	58·0 ± 8·0	40·5 ± 14·0	5·0 ± 1·5
Range	49–71	24–71	3·5–6·5

have shown that, with the specific activity employed, the average background count of control specimens remains below three. For the calculation of the mean number of SLC and the mean number of grains/SLC 1000 cells and 100 cells were counted, respectively. The mean number of SLC and the mean number of grains per SLC following an exposure to 2 MEDs of UV-B served as a baseline for comparing the amount of UDS induced in the experiments. Cells with very low grain numbers (below 3) corresponding to background label of non-irradiated controls and heavily labelled cells undergoing S-phase were not counted. From each biopsy specimen 10–15 randomly selected sections were evaluated. The data obtained from the experimental and control areas were compared by statistical analysis (t-test and analysis of variance).

RESULTS

UV-B vs UV-A

When UV-B was compared with approximately equally erythemogenic doses of UV-A, UV-A proved to induce from 50 to 60% of the amount of UDS produced by UV-B (Table 4), regardless whether 2 or 3 × MED of UV-A or UV-B were used (Fig. 1a,b). The difference in the number of SLC and of grains per SLC in UV-B and UV-A irradiated skin was highly significant ($P < 0{\cdot}01$).

TABLE 4. UV-B vs UV-A

	SLC/1000 (means)		Grains/cell (means)	
UV-B (2 × MED)	593	$P < 0{\cdot}01$	7·24	$P < 0{\cdot}01$
UV-A (2 × MED)	338		4·4	
UV-B (3 × MED)	668	$P < 0{\cdot}01$	7·0	$P < 0{\cdot}01$
UV-A (3 × MED)	336		4·5	

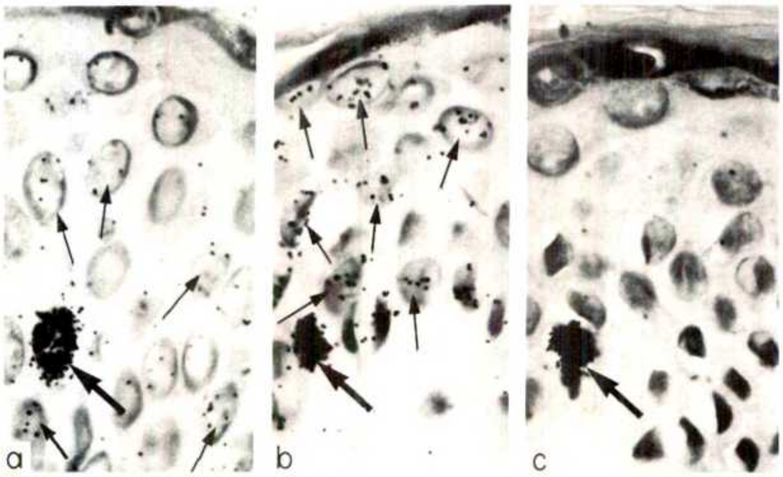

FIGURE 1. Autoradiographs demonstrating ^{3}H-thymidine sparse label over nuclei of human epidermal cells (arrows). Thick arrows indicate densely labelled cells undergoing S-phase. (a) After 2 MEDs of UV-A. (b) After 2 MEDs of UV-B. (c) Non-irradiated control.

TABLE 5. PUVA vs UV-B

	SLC/1000 (means)	Grains/cell (means)
PUVA (2 × MPD)	–*	–
UV-B (2 × MED)	517	7·3
After 24 h		
PUVA (2 × MPD)	–*	–
UV-B (2 × MED)	–*	–

* Label indistinguishable from non-irradiated control.

PUVA vs UV-B

High dose (2MPDs) PUVA did not induce UDS (Table 5); neither the number of SLC nor the mean grains per SLC were different from the background label of non-irradiated control specimens. This was also true for specimens biopsied 24 h after irradiation, at which time no UDS could be observed in both PUVA and UV-B treated areas (Table 5).

UV-B vs UV-B plus UV-A

The combination of one quarter MED of UV-A and 2 × MED of UV-B, delivered in either sequence did not significantly change the amount of UDS as compared with that induced by UV-B alone (Table 6).

TABLE 6. UV-B vs UV-B plus UV-A

	SLE/1000 (means)		Grains/cell (means)	
UV-B (2 × MED)	665	n.s.	7 9	n.s.
UV-A → UV-B (1/4 MED) (2 × MED)	650		7·2	
UV-B → UV-A (2 × MED) (1/4 MED)	626		6 4	
UV-A (1/4 MED)	–*		–	

* Label indistinguishable from non-irradiated control.

PUVA vs PUVA plus UV-B

The combination of 1 MPD of PUVA with 2 MEDs of UV-B in either sequence did not lead to a significant increase or decrease of UDS as compared with UV-B alone (Table 7).

Controls

In the non-irradiated control specimens a faint background label was present. This consisted of a maximum of sixty SLC/1000 cells with grain numbers not exceeding 3 per SLC (Fig. 1c).

TABLE 7. PUVA vs PUVA plus UV-B

	SLC/1000 (means)		Grains/cell (means)	
PUVA (1 MPD)	–*		–	
PUVA ⟶ UV-B (1 MPD) (2 × MED)	761	n.s.	8·8	n.s.
UV-B ⟶ PUVA (2 × MED) (1 MPD)	749		8·5	
UV-B (2 × MED)	735		7·2	

* Label indistinguishable from non-irradiated control.

DISCUSSION

According to present concepts sparse autoradiographic labelling is considered a measure for unscheduled DNA synthesis (UDS) indicating excision repair of UV-induced cyclobutylpyrimidine dimers and other endonuclease-sensitive sites (Epstein, Fukuyama & Epstein, 1969; Pathak, Krämer & Güngerich, 1972; Willis *et al.*, 1973). Below the saturation dose of UV (and/or other DNA-damaging agents) (for references see Ahmed & Setlow, 1979; Brown *et al.*, 1979) the amount of excision repair can be quantitatively correlated with the UV dose and the amount of UDS may thus allow estimation of the magnitude of UV-induced DNA damage. Repair of these DNA lesions may be performed in the normal cell by at least three different mechanisms: photoreactivation, post-replication repair and excision repair (Setlow & Setlow, 1972; Hart, Hall & Daniel, 1978). In mammalian cells the excision repair appears to be the most important mechanism (Cleaver, 1974). Most of the available information on DNA repair in mammalian skin concerns repair of lesions after UV-C or UV-B irradiation, and only sparse data exist on the effects of UV-A and/or PUVA. The goal of the present study was therefore to compare the amount of excision repair as expressed by unscheduled DNA synthesis after biologically comparable (i.e. equally erythemogenic) doses of narrow-banded UV-A and UV-B and PUVA, and to investigate the effects of combined exposures of the respective UV wavebands on UDS in the skin.

UV-A as compared to UV-B

Willis *et al.* (1973) have found only minimal amounts of UDS when skin was irradiated with UV-A alone. Their findings, however, are difficult to interpret because they did not apply comparable erythemogenic doses of UV-A and UV-B and their radiometric information does not permit comparison with our results. In our study, UV-A induced 50–60% of UDS produced by equally erythemogenic doses of UV-B. This observation is in keeping with other data indicating that UV-A is photobiologically active in skin. UV-A is erythemogenic (Pathak *et al.*, 1972; Parrish *et al.*, 1974; Willis & Cylus, 1977; Kaidbey & Kligman, 1979) and although there is some disagreement about the mechanism by which it enhances the UV-B sunburn reaction (Willis *et al.*, 1973; Parrish *et al.*, 1974; Ying *et al.*, 1974; Kaidbey & Kligman, 1975; Spiegel *et al.*, 1978) there is no doubt that such enhancement occurs. Because of its deeper penetration into the skin, UV-A is thought to be involved in actinically induced ageing of skin (Kligman, 1974) and, although conclusive data are not yet available, UV-A has been suspected

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of enhancing UV-induced carcinogenesis (Forbes, Davies & Urbach, 1978). Erythemogenic doses of UV-A produce morphological changes within the epidermis which are qualitatively similar to UV-B-induced damage (Stern & Urbach, 1972; Parrish *et al.*, 1976; Willis & Cylus, 1977; Kumakiri *et al.*, 1977; Kaidbey & Kligman, 1979; Rosario *et al.*, 1979) and even doses below the erythema threshold yield histochemical and ultrastructural alterations in the Langerhans cell system (Aberer *et al.*, 1980). Furthermore, in a recent study, Andrews (1980) was able to demonstrate lethal (or growth inhibiting) effects of UV-A in repair deficient xeroderma pigmentosum cells but not in normal cells. The latter observation suggests that UV-A produces DNA lesions which require the same repair system as lesions induced by UV-B and UV-C.

UV-B as compared to UV-B plus UV-A

The rationale of this part of the study was to test whether UV-A enhances sunburn damage also at the molecular level. One quarter MED of UV-A was thought an appropriate dose to combine with 2 MEDs of UV-B, since this dose did not induce measurable amounts of UDS when applied alone and lies within the range used previously for photoaugmentation experiments (Willis *et al.*, 1973; Kaidbey & Kligman, 1975). An increase of UDS could have been interpreted as a photoaugmentative effect of UV-A. Clinically UV-A has been shown to potentiate the UV-B induced sunburn reaction by enhancing the delayed erythema. However, in this study no changes in the amount of UV-B induced UDS were observed regardless of whether UV-A was applied before or after UV-B. This may be in accordance with the findings of Kaidbey, Grove & Kligman (1979) who were unable to demonstrate 'photoaugmentation' when sunburn cells in human epidermis were used as criterion for evaluation. Erythema and other UV-induced reactions of skin may thus be mediated by different mechanisms and photoaugmentation is apparently reflected only by the erythematous response.

Our findings are of interest also from another point of view. UV-A has been shown to strongly inhibit the excision repair of UV-C lesions in bacterial DNA (Tyrrell & Webb, 1973) and we had thus expected to see a decrease of UV-B induced UDS when adding UV-A to the irradiation protocol. However, at least with the moderate UV-A doses used in this study, no obvious alteration of the excision repair was observed.

PUVA as compared to UV-B and PUVA plus UV-B

Psoralens are able to form monofunctional and bifunctional adducts (interstrand crosslinks) with DNA when irradiated with UV-A (reviewed by Rodighiero & Dall'Acqua, 1976). The absence of UDS after the treatment of skin with PUVA was unexpected, since there exists recent evidence (Kaye, Smith & Hanawalt, 1980) that PUVA-induced monofunctional DNA lesions are subject to repair mechanisms similar to the classical excision repair in human cells. These authors have shown that with cultured human fibroblasts the enzymatic pathway of psoralen monoadduct repair appears to share at least one step with the pyrimidine dimer repair system. Similar findings have been presented very recently by Gruenert & Cleaver (1980). In earlier experiments Baden *et al.* (1972) had noted small amounts of UDS in UV-A irradiated methoxsalen-sensitized human fibroblasts and Swanbeck *et al.* (1977) observed minimal amounts of UDS in human skin after PUVA treatment. However, these findings may have been due to contaminating traces of UV-B which are present in all UV-A and PUVA light sources that are not appropriately filtered. On the other hand, Epstein & Fukuyama (1975) and, more recently, Bishop (1979) and Bioulac *et al.* (1980) have failed to demonstrate UDS by autoradiography in mammalian epidermis when using filtered UV-A sources, which is in accord with our findings. Obviously, the dose of UV-A, which is adequate for a phototoxic reaction, is too low to induce measurable UDS. Experimental evidence indicates that prolonged exposure to UV-A in the presence of psoralens alters the proportion of monoadducts to crosslinks in favour of the latter (Rodighiero & Dall'Acqua, 1976). It thus seems possible that with PUVA as utilized for therapeutical purposes, interstrand crosslinks, which presumably are not repairable via the classical excision repair, occur in human skin, and repair of very small amounts of monofunctional adducts is below the resolution provided by the method employed. Our failure to detect UDS 24 h after PUVA treatment suggests also the absence of a measurable late effect on UDS.

There are three possible explanations for the absence of UDS in PUVA-treated skin. First, the majority of PUVA induced DNA lesions consist of crosslinks which cannot be repaired by mechanisms employing UDS. Secondly, excision repair of PUVA-induced DNA lesions may be operative at times after irradiation not examined in this study. Thirdly, PUVA-induced crosslinks with DNA may block the excision repair system and therefore may suppress subsequent resynthesis of DNA. If this third hypothesis were true one should expect a decrese in UV-B induced UDS as well. The findings of the present investigation indicate that, within our experimental system, PUVA does not interfere with the repair of UV-B lesions. Moreover, we were unable to demonstrate a photoaugmentative effect of PUVA for UV-B induced DNA damage.

CONCLUSIONS

High doses of UV-A induce a considerable amount of UDS in human skin which suggests that pyrimidine dimers and other DNA damage that had occurred is repairable by excision repair. As the formation of dimerization bears the risk of mutagenesis, long-term and high-dose UV-A exposure (e.g. for cosmetic purposes) may certainly not be as harmless as is generally assumed. Combined exposures of UV-A and UV-B, within the dose ranges used in this study do not lead to quantifiable changes in the amount of UDS as compared with UV-B alone and may thus, perhaps, not increase DNA damage. This could be interpreted as a possible argument against a hypothetical photoaugmentative effect of UV-A at the molecular level. On the other hand, UV-A alone does induce UDS at more than half the rate of equi-erythemogenic UV-B doses, and suberythemogenic UV-A and UV-B doses appear to be additive for erythema production. Dose reponse studies of UDS as a function of the MED are under way to clarify whether higher doses of UV-A and UV-B may add and perhaps reach a saturating level for the repair capacity in skin.

PUVA treatment of human skin apparently does not give rise to the formation of detectable DNA lesions, which can be repaired by a UDS system which indirectly suggests a prevalence of interstrand crosslink generation. On the other hand, these PUVA induced crosslinks apparently do not inhibit the repair activity following UV-B exposure. This is of clinical relevance because most of the PUVA irradiation units used for the treatment of skin disorders deliver small amounts of UV-B, which, with prolonged exposures, can accumulate to considerable doses. In addition, most patients cannot avoid some sun exposure of the face before or after their treatments, or may not be instructed to avoid sunlight prior to PUVA treatment.

The data obtained in the present study can refer only to the bands of UV-radiation used. Whether the conclusions drawn from these data are valid for other wave lengths, or whether other phenomena, or competing or supporting photobiological events, occur when very broadband radiation is employed remains to be clarified by further investigation.

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Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol/UV-B

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SUMMARY

Cell mediated immune reactivity was studied in eighty-five psoriasis patients and twenty-five healthy controls by an improved (quantitative) method for measuring contact hypersensitivity to 2,4 dinitrochlorobenzene (DNCB). Patients were sensitized with 500 μg DNCB, the lowest dose found to sensitize all healthy subjects. Responses to epicutaneous challenge with a series of concentrations of DNCB were measured as volumes calculated from diameter and thickness and the various groups were compared by the differences in the log dose-response curves. Patients with untreated psoriasis were less responsive than healthy controls; responses were less still in patients treated with dithranol/UV-B/tar and they were least of all in patients treated with photochemotherapy with 8-methoxypsoralen and UV-A (PUVA), particularly in those who pigmented least. Sensitization and challenge at different stages of treatment showed that both induction and elicitation of sensitization were impaired by PUVA. The possible relationship of these changes to DNCB metabolism, Langerhans cell damage and a decrease in circulating T cells is discussed. Although the clinical significance of the findings is unknown, treatment with dithranol/UV-B/tar has proved safe over many years of use.

Reduced T lymphocyte reactivity has been observed in patients with psoriasis after photochemotherapy with 8-methoxypsoralen and long wavelength ultraviolet irradiation (PUVA) (Kraemer & Weinstein, 1977; Friedmann & Rogers, 1980). To investigate the significance of this observation we have studied contact hypersensitivity induced and elicited by topical 2,4-dinitrochlorobenzene (DNCB) in patients receiving PUVA therapy, and compared it with the response in untreated patients and in patients receiving treatment with tar, UV-B irradiation and dithranol (the Ingram regimen) (Ingram, 1954).

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PATIENTS AND METHODS

Sensitization and challenge

In preliminary experiments, 500 μg of DNCB was found to be the lowest dose that sensitized 100% of normal subjects, and this dose (100 μl of a 0·5% solution of DNCB in acetone) was therefore applied to the volar aspect of the left forearm within a circle 3 cm in diameter. After it had dried, the area was covered with a polythene patch and occluded with Elastoplast for 48 h. Three weeks after sensitization, patients were challenged with three concentrations of DNCB applied to the right forearm. Skinfold thickness was first measured at three sites with Harpenden calipers from which one spring was removed to reduce compression (T1). The reproducibility of readings and observer error has been established previously (Cook & Shuster, 1980). Then 10 μl of three different dilutions of DNCB, giving doses of 12·5, 6·25 and and 3·125 μg, were pipetted onto 'A1-test' patch test felts which were applied to the three sites and held in place for 24 h with adhesive tape. The responses were measured at 48 h as diameter of induration and skinfold thickness (T2). The patch test response was expressed as volume, calculated as

$$\Pi r^2 \times \tfrac{1}{2}(T2 - T1)$$

Subjects

In order to establish whether responsiveness to DNCB is altered by psoriasis or by its treatment, the following groups were studied (Table 1): healthy volunteers; patients with untreated psoriasis; patients with psoriasis undergoing treatment with the Ingram regimen (daily tar bath, UV-B irradiation and dithranol); patients with psoriasis receiving PUVA therapy thrice weekly (Rogers *et al.*, 1979). Patients receiving treatment with the Ingram regimen were both sensitized and challenged during treatment. Patients to be treated with PUVA were subdivided into three groups as follows in order to distinguish between an effect of PUVA on the induction of sensitivity and on its expression: patients sensitized 1–4 weeks before PUVA therapy and challenged while receiving PUVA; patients both sensitized and challenged while on PUVA; patients sensitized on PUVA but challenged 7–10 days after the completion of treatment. The skin type of patients treated with PUVA was graded I–V on the basis of burn and tan responses to solar irradiation (Rogers *et al.*, 1979).

TABLE 1.

Subjects	Treatment	Number		Age: mean ± s.d.
		Males	Females	
Healthy	None	8	17	44 ± 18
Psoriatic	None	10	9	38 ± 19
	Ingram regimen	11	5	47 ± 19
	PUVA: sensitized before, and challenged during treatment	11	6	42 ± 18
	PUVA: sensitized and challenged during treatment	13	8	40 ± 15
	PUVA: sensitized during, and challenged after treatment	9	3	47 ± 14

Analysis of results

Results were subjected to analysis of variance and a log dose-response curve was computed for each group. Skin type and patch test response were examined for correlation by the Kruskal Wallis analysis of variance.

RESULTS

The log dose-response curves are shown in Fig. 1. Patients with untreated psoriasis were significantly less responsive to DNCB than normal subjects ($P < 0{\cdot}01$). Patients on the Ingram regimen were less reactive than untreated psoriatic patients ($P < 0{\cdot}05$) and the reduction in reactivity was even more marked in patients sensitized and tested during PUVA treatment ($P < 0{\cdot}01$). Patients sensitized before PUVA and challenged during PUVA gave smaller responses than untreated patients; patients sensitized during PUVA but challenged 7–10 days after PUVA gave still smaller responses, which were comparable with those of patients both sensitized and challenged during PUVA therapy.

When responses from patients both sensitized and challenged while receiving PUVA were correlated with skin type, DNCB reactivity was found to be greatest in patients who tan most readily; the correlation was highly significant ($P < 0{\cdot}001$). A similar trend was apparent in the other groups treated with PUVA and in the untreated patients, but the correlation failed to reach significance.

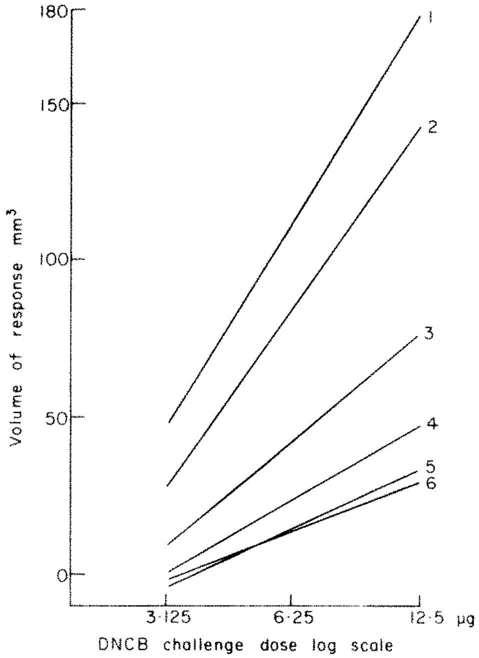

FIGURE 1. Responses to DNCB challenge in patients with psoriasis and controls. Computed dose response curves are shown for (1) healthy controls, (2) patients with untreated psoriasis, (3) psoriatic patients treated with tar/UV-B/dithranol, (4) patients sensitized before treatment with PUVA and challenged while receiving PUVA, (5) patients sensitized on PUVA but challenged 7–10 days after completion of treatment, and (6) patients both sensitized and challenged while on PUVA.

DISCUSSION

The methodology of this study differs from that used previously. Other workers (Obalek, Haftek & Glinski, 1977) have used 2000 μg to induce sensitization, after the method of Catalona, Taylor & Chretien (1972). Since this is well above the 500 μg which we find to be the lowest dose required to sensitize 100% of normal people, standard sensitization procedures could fail to detect a reduction in responsiveness. This is apparent in the recent study of Strauss *et al.* (1980) in which the impaired response in untreated psoriatics was not detected. We have also augmented the resolving power of the method by measuring patch test responses as volume instead of using a subjective grading procedure and finally, and most importantly, by establishing dose-response curves. Thus the differences we found between the dose-response curves for the various groups, particularly between untreated patients with psoriasis and healthy controls, were not apparent from analysis of responses to any single dose of DNCB. The reduced responsiveness to DNCB of patients with untreated psoriasis is in keeping with the findings of Epstein & Maibach (1965) and may reflect an immunological defect. It might also be due to an underlying metabolic defect: thus DNCB is an aryl hydrocarbon and it may require conversion in the skin to a more allergenic compound by epidermal aryl hydrocarbon hydroxylase, the activity of which is reduced in psoriasis (Chapman, Shuster & Rawlins, 1979; Shuster *et al.*, 1980).

Patients sensitized and tested during treatment with PUVA or the Ingram regimen showed diminished reactivity to DNCB. The slopes of the dose-response curves were significantly different from that for untreated patients which suggests qualitative as well as quantitative differences in the inhibition by different forms of treatment. Confirmation of this would require full dose-response curves to ensure comparison was being made in the linear part of each.

We studied the mechanism of the inhibitory effect of PUVA by sensitizing and challenging patients at different times in relation to treatment. Patients sensitized before PUVA and challenged during PUVA were significantly less responsive than untreated patients with psoriasis, showing that PUVA inhibits the elicitation of DNCB sensitivity. Patients sensitized during PUVA and challenged 7–10 days after treatment had stopped were also less reactive than untreated patients, demonstrating that PUVA also impairs primary sensitization. Although the impairment of sensitization by PUVA was significantly greater than impairment of elicitation, we cannot exclude the possibility that this was due to the different cumulative doses of PUVA and other uncontrolled variables. These unknown factors may also explain why an additive effect was not found when both sensitization and elicitation were carried out during PUVA treatment.

The finding of an inhibitory effect of PUVA on expression of sensitization accords with reports of reduced patch test responses during PUVA therapy to common allergens in patients with pre-existing contact hypersensitivity (Thorvaldsen & Volden, 1980) and to topical mustine hydrochloride in patients with mycosis fungoides (Volden, Molin & Thomsen, 1978). These changes may be due to the decrease in T lymphocyte numbers and responses induced by PUVA (Haftek *et al.*, 1979; Friedmann & Rogers, 1980).

Langerhans cells, which are involved in antigen presentation in contact hypersensitivity, are damaged by UV irradiation (Toews, Bergstresser & Streilein, 1980), and this may have contributed to the impairment of reactivity to DNCB which we found in patients treated with PUVA or the Ingram regimen. Furthermore, protection of Langerhans cells or lymphocytes by melanin could account for the observation that DNCB sensitivity was suppressed least in patients who tan most readily.

Although we have observed an impairment of immune reactivity in patients with untreated psoriasis, which was greater in patients treated with the Ingram regimen and greater still in patients treated with PUVA, the consequences of such impairment are not clear. If this loss of immune reactivity has pathological importance, then patients with psoriasis would be expected to show increased susceptibility to infections and the development of tumours, and increased susceptibility should be proportionately greater in the two groups receiving treatment. However, there is no evidence that this is so in untreated patients for either infections or for tumours. Indeed it has been suggested that people with psoriasis may be less likely to develop skin and other cancers (Kocsard, 1976; Shuster, Chapman & Rawlins, 1979). There are no reports of an increased frequency of skin tumours in patients treated with tar or dithranol and UV-B. Although a carcinogenic effect of PUVA has been postulated (Bridges & Strauss, 1980), the one report of an increased incidence of tumours in patients treated with PUVA (Stern *et al.*, 1979) is open to the criticism that controls were retrospective and were not subjected to the same detailed examination as the treated patients.

Strauss *et al.* (1980) also found impaired DNCB responsiveness in patients with psoriasis receiving PUVA, and concluded that PUVA might promote skin cancer by impairment of immune surveillance. However, since they omitted the necessary control group of patients with psoriasis receiving conventional therapy, in whom there is no question of an increased risk of cancer, their conclusion is unjustified.

It is likely, therefore, that the immune system operates with a large functional reserve and only when it has been grossly impaired, by congenital deficiency or immunosuppressive therapy, are such complications common. The degree of impairment of immune reactivity that we have observed is small by comparison, as was the difference between that produced by PUVA and dithranol/tar/UV-B. Thus, unless further studies show additional quantitative or qualitative differences between the two therapies (such as a more persistent immunosuppression or a more specifc impairment of effector mechanisms that control infection and tumour growth) together with their clinical sequelae, we must conclude that the immunological defects which have been detected indicate sensitivity of the methods rather than seriousness of the results.

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Studies on the plasma membrane of normal and psoriatic keratinocytes.

4. Characterization of glycoconjugates

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SUMMARY

A substantial proportion (20–50%) of radioactive sugar incorporated into glycoconjugates by normal human keratinocytes was soluble in chloroform-methanol; using (^{14}C)-galactose as precursor about half of this fraction was neutral lipid. The incorporation of labelled sugars into the lipid fraction was consistently increased in keratinocytes derived from psoriatic lesions.

The abnormality in fucose metabolism which we reported previously has been confirmed. In particular we have shown that the molecular weight of fucosylated glycopeptides appears to be abnormally high in the untreated psoriatic lesion, possibly reflecting an increased degree of branching. In psoriatic uninvolved epidermis and in treated psoriatic lesions the situation is reversed, the molecular weight being significantly lower than normal.

The uptake of labelled sugars by keratinocytes and their subsequent incorporation into glycoconjugates (glycoprotein, glycolipid and proteoglycan) have already been described (Roelfzema *et al.*, 1981). Certain differences between normal and 'psoriatic' keratinocytes were observed, in particular in the metabolism of fucose.

We have now studied the labelled glycoconjugate pool in greater detail in order to define this abnormality more sharply. Here we report its separation into lipid and non-lipid fractions and the partial characterization of these components.

MATERIALS AND METHODS

Materials

Sephadex G50 was obtained from Pharmacia (Uppsala, Sweden), silica gel from Merck (Darmstadt, Germany) and papain (type III, 2 × cryst.), fetuin, transferrin and thyroglobulin

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from the Sigma Chemical Co., Ltd (St Louis, Miss., U.S.A.). Authentic samples of lipids were provided by Applied Science Laboratories, Inc., Philadelphia, U.S.A. All other chemicals were as described previously (Roelfzema *et al.*, 1981). The selection of healthy volunteers and psoriatic patients, method of biopsy and preparation of keratinocyte suspensions were as reported by Gommans *et al.* (1979). For certain experiments additional biopsies were obtained from psoriatic lesions which had been treated with coal tar and dithranol (Ingram, 1954) for 1–3 days prior to excision.

Labelling and isolation procedures

Incubation of cells with radioactive sugars and the subsequent isolation of a 'TCA-insoluble sugar' pellet was as before (Roelfzema *et al.*, 1981) using a 3 h or 22 h labelling period.

The general protocol for fractionation of the TCA-insoluble material is summarized in Fig. 1. The pellet was extracted with 2 × 1 ml portions of chloroform-methanol (2:1 v/v) according to Folch, Lees & Sloan-Stanley (1957). After drying at 60°C, the residue was resuspended in 0·5 ml of 0·2 M acetate buffer (pH 6·5) containing 2 mg/ml cysteine. Papain (3 mg) was added, and the mixture incubated for 24 h at 60°C. The hydrolysate was cooled to 4°C and centrifuged. The supernatant, which contained labelled glycopeptides and the sulphated glycosaminoglycans, was further fractionated by gel filtration on Sephadex G50.

In order to avoid the possibility that labelled dolichol derivatives were present in large amounts, the residue obtained after chloroform-methanol extraction was extracted again with chloroform-methanol-water (10:10:3 by volume). Using keratinocytes which had been labelled for 22 h, this extract contained only 3–5% of the total TCA-insoluble radioactivity regardless of whether the cells were derived from healthy skin or psoriatic lesions. This procedure was therefore omitted in the following experiments.

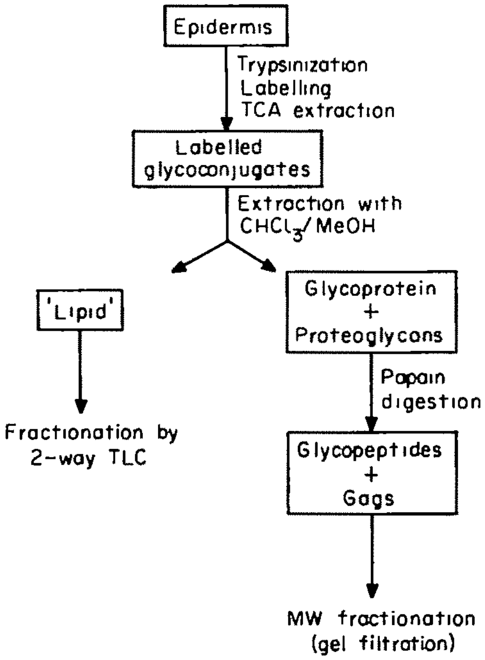
Epidermis
Trypsinization
Labelling
TCA extraction
Labelled glycoconjugates
Extraction with $CHCl_3$/MeOH
'Lipid'
Fractionation by 2-way TLC
Glycoprotein + Proteoglycans
Papain digestion
Glycopeptides + Gags
MW fractionation (gel filtration)

FIGURE 1. General protocol for fractionation of labelled glycoconjugates.

Gel filtration

In general samples of 0·5 ml were applied to a Sephadex G50 column which had been equilibrated with 0·2 M pyridine-acetate buffer (pH 5·0) and were eluted with the same buffer. The column dimensions were 2·5 × 32 cm, the flow-rate was 35 ml/h, and 2·25 ml fractions were collected. In all experiments UV monitoring was used to establish the position of added marker proteins. In addition, 'standard' glycopeptide mixtures were prepared by papain digestion of fetuin, desialyzed fetuin, transferrin and thyroglobulin, and the elution profiles recorded.

Thin-layer chromatography (TLC)

Two-dimensional TLC was performed according to Broekhuyse (1968). Silica gel HR (containing 3% alkaline magnesium silicate) was used as adsorbant. The chloroform-methanol extracts were evaporated to dryness, taken up in benzene-methanol (4:1 v/v) and applied to the plates. After development with chloroform-methanol-7 M ammonia (90:54:11 by volume) the chromatograms were dried *in vacuo* for 1 h over H_2SO_4 and developed in the second direction with chloroform-methanol-acetic acid-water (90:40:12:2 by volume). Both solvent systems contained 50 mg/l of butylated hydroxytoluene. Lipids were detected with iodine vapour. Standards included phosphatidylcholine, phosphatidylethanolamine and cholesterol.

Measurement of radioactivity

In general this was as described previously (Roelfzema *et al.*, 1981). Samples of silica gel were scraped from thin-layer plates, suspended in 1 ml water and mixed with 10 ml 'Aquasol' for scintillation counting. Counting efficiency was about 80% for ^{14}C and 45% for ^{3}H.

RESULTS

Fractionation into lipid and non-lipid components

Preliminary experiments, using normal human keratinocytes and (^{14}C)-galactose, showed that the percentage of the total TCA-insoluble radioactivity which extracts into chloroform-methanol (i.e. lipid) rises from 22 ± 2% (n = 5) after a 3 h labelling period to 40 ± 2% (n = 12) after 22 h labelling. The latter period was selected for subsequent experiments; four different labelled sugars were investigated and the behaviour of 'psoriatic' keratinocytes compared with controls. It is seen from Fig. 2 that, regardless of the sugar employed, the percentage of radioactivity in the lipid fraction was the same for 'uninvolved' psoriatic epidermis and controls, but was markedly increased using keratinocytes derived from psoriatic lesions.

In all keratinocyte preparations some large, polygonal cells could be observed which still retained nuclei (Gommans *et al.*, 1979). Preparations derived from healthy epidermis contained relatively few of these cells (usually about 1% of the total population), but in preparations from psoriatic lesions these accounted for up to 50% of the total. Remarkably, we observed that when using (^{14}C)-galactose the percentage of radioactivity in the lipid fraction increased as a function of the percentage of the 'large' cells in the keratinocyte preparation (Fig. 3). No such correlation was apparent in the cases of the other three sugars.

TLC of the lipid fraction

Two samples each of the lipid extracts derived from normal, psoriatic lesion and psoriatic uninvolved epidermis (all labelled with ^{14}C-galactose) were combined in order to obtain sufficient material for visualization of the individual components. Examination of chromato-

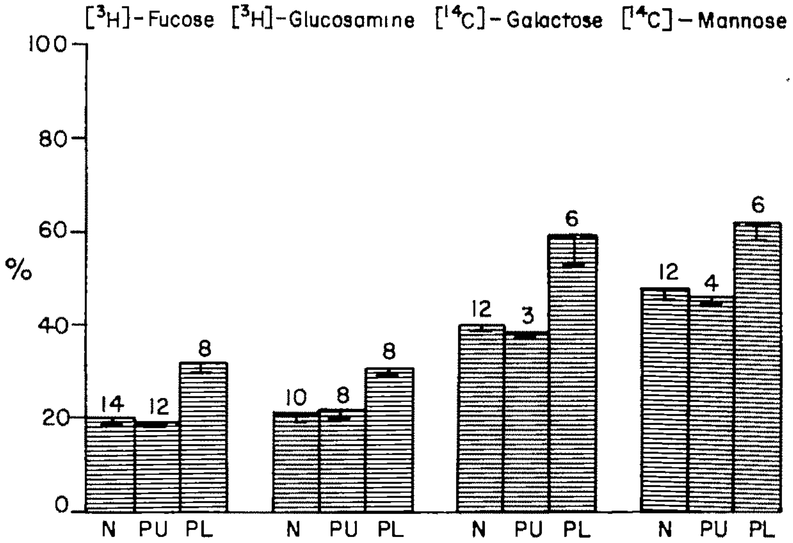

FIGURE 2. Percentage of radioactivity extracted from labelled glycoconjugates by chloroform-methanol. Symbols are as follows: N = normal epidermis; PU = uninvolved epidermis from psoriatic patients; PL = psoriatic lesion.

grams treated with iodine vapour revealed a substantial proportion of material remaining at the origin, a single major component and five to six minor components.

The major component co-ran with cholesterol (i.e. near the solvent fronts) and was designated as neutral lipid. The R_F values of the various minor components indicated that these were probably phospholipids, but positive identification of the individual compounds was not made. An area corresponding to each spot was scraped off and the radioactivity measured. The remaining plate was divided into a grid of sixteen squares and the activity of each was determined; in no case did this exceed 0·5% of the total activity.

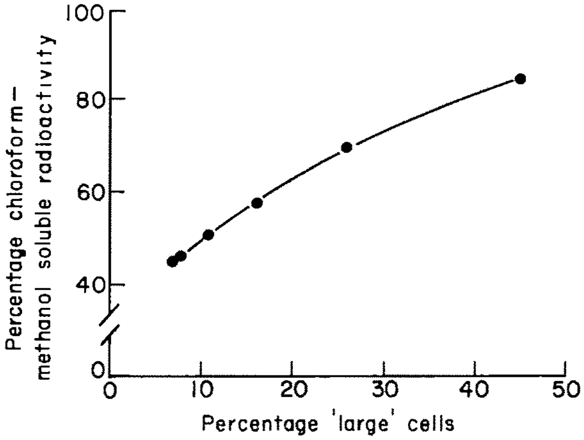

FIGURE 3. Percentage of radioactivity extracted from labelled glycoconjugates by chloroform-methanol as a function of the percentage of 'large' cells in the preparation. All specimens were from psoriatic lesions and were labelled with (^{14}C)-galactose.

TABLE 1. TLC of lipid fraction. Keratinocytes were labelled for 22 h with (^{14}C)-galactose

Specimen	Percentage radioactivity		
	Origin	Neutral lipid	Minor components
Normal epidermis	21	51	28
Psoriatic 'uninvolved' epidermis	19	46	35
Psoriatic lesion	22	50	28

Both the percentage of neutral lipid (Table 1) and the 'patterns' of activity of the minor components were very similar for all three specimens.

Gel filtration of glycopeptides and glycosaminoglycans

The molecular weight distributions of the glycopeptides solubilized by papain, using normal human keratinocytes as starting material, are illustrated in Fig. 4. In general four peaks can be distinguished, although their relative contributions are very dependent on the particular sugar used for labelling. Calibration with standards of known molecular weight and comparison with

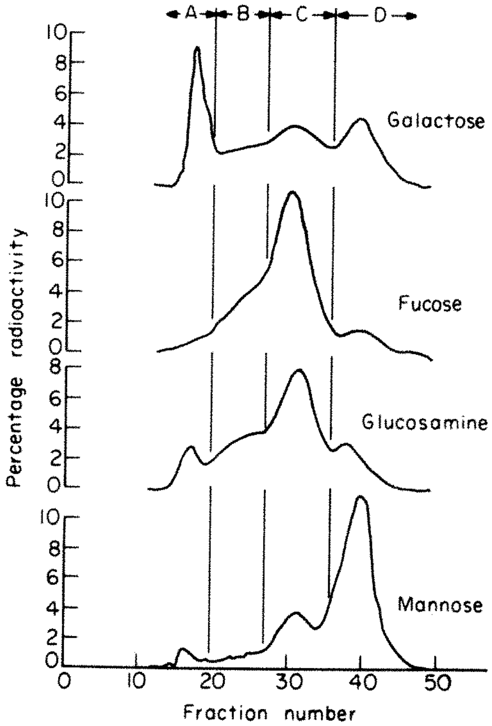

FIGURE 4. Molecular weight distribution of glycopeptides solubilized by papain, using normal human keratinocytes as starting material. Curves were prepared by averaging the data for all normal specimens using a TI 59 microcomputer.

TABLE 2. Percentage distribution of radioactivity into fractions A–D. Symbols are as follows: N = normal epidermis; PU = psoriatic uninvolved epidermis; PL = psoriatic lesion; TPL = treated psoriatic lesion. Numbers of specimens are in parenthesis. Values are ± s.e.mean.

Sugar	Specimen	Fraction A	Fraction B	Fraction C	Fraction D	Ratio C/B
Galactose	N (5)	24·8 ± 2·4	17·3 ± 0·4	30·1 ± 0·9	27·0 ± 1·9	1·8 ± 0·1
	PU (3)	21·4 ± 4·6	19·1 ± 1·3	30·8 ± 1·4	28·1 ± 2·6	1·6 ± 0·1
	PL (4)	31·0 ± 11·4	15·2 ± 2·0	27·2 ± 4·0	26·2 ± 5·7	1·8 ± 0·1
Fucose	N (7)	3·9 ± 0·6	23·8 ± 0·8	61·3 ± 1·8	10·1 ± 2·3	2·6 ± 0·1
	PU (9)	2·3 ± 0·3	16·9 ± 1·1	69·4 ± 1·4	10·8 ± 1·0	4·3 ± 0·4
	PL (5)	9·9 ± 2·4	30·1 ± 3·1	43·9 ± 4·1	13·9 ± 4·6	1·6 ± 0·2
	TPL (5)	2·8 ± 0·4	19·8 ± 1·7	66·8 ± 1·2	10·1 ± 1·4	3·5 ± 0·4
Glucosamine	N (7)	10·3 ± 1·6	21·7 ± 0·6	51·5 ± 1·2	16·2 ± 0·8	2·4 ± 0·1
	PU (5)	9·8 ± 1·6	16·8 ± 1·7	52·1 ± 1·0	20·7 ± 2·0	3·2 ± 0·3
	TPL (5)	5·7 ± 0·3	16·9 ± 1·4	48·9 ± 1·9	27·2 ± 2·2	3·0 ± 0·2
Mannose	N (4)	4·2 ± 1·6	5·8 ± 0·2	26·3 ± 2·8	62·6 ± 2·3	4·5 ± 0·4
	PU (3)	3·8 ± 1·8	5·7 ± 0·2	23·2 ± 1·5	65·1 ± 0·5	4·1 ± 0·2
	PL (2)	2·8	4·8	22·7	67·0	5·0

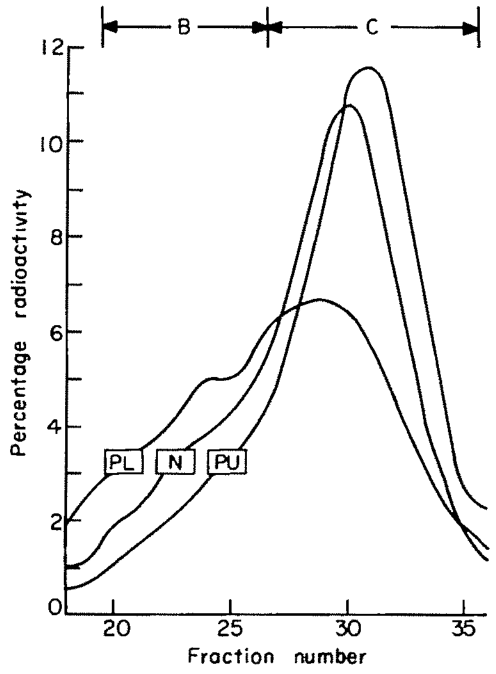

FIGURE 5. Elution profiles of glycopeptides, following labelling with (^{3}H)-fucose, showing the shift to higher mol. wt in the psoriatic lesion and to lower mol. wt in the psoriatic uninvolved skin. Curves were prepared by averaging the data from all (^{3}H)-fucose labelled specimens. Symbols are as in Fig. 2.

published data (Warren, Buck & Tuszynski, 1978; Krusius *et al.*, 1974; Kobata, 1979) indicate that the composition of these peaks is as follows:

Fraction A glycosaminoglycans and/or O-glycosidic glycopeptides (mucin-type).
Fraction B N-glycosidic glycopeptides: complex type with four or more chains.
Fraction C N-glycosidic glycopeptides: complex type with two chains and high mannose-type chains.
Fraction D oligosaccharide fragments.

N-glycosidic glycopeptides, of the complex type carrying three chains (obtained by papain digestion of fetuin), were distributed equally between fractions B and C. In addition it was established that removal of the terminal sialic acid group from glycopeptides resulted in a shift to a fraction of lower mol. wt.

To facilitate quantitative comparison of the various specimens, the areas under each peak were evaluated; these data are summarized in Table 2. It is seen that the most striking changes in the psoriatic keratinocytes occur in the distribution of fucose. In particular, the ratio C/B is significantly reduced in untreated psoriatic lesions ($P < 0{\cdot}01$), whereas in both 'uninvolved' psoriatic epidermis and in lesions after 1–3 days therapy this ratio is increased ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively). The actual elution profiles of (^{3}H)-fucose from the various specimens are illustrated in Fig. 5; the shift to higher mol. wt for untreated lesions and to lower mol. wt for the 'uninvolved' psoriatic epidermis is evident.

No correlation was observed between the mol. wt distributions and the percentage of 'large' cells in the individual specimens.

DISCUSSION

Our results make it clear that normal human keratinocytes incorporate a substantial proportion of labelled sugar into the lipid fraction. This finding is in line with the report of Speak & White (1978), who studied the fate of mannose in mammary gland. These workers showed that up to 20% of the radioactivity taken up by the cells was soluble in chloroform-methanol, and reported that 80–90% of this fraction was neutral lipid. The consistently higher percentage of radioactivity found in the chloroform-methanol fraction using keratinocytes from psoriatic lesions (Fig. 2) may well indicate a real increase in the synthesis of neutral or phospholipids in this disease, an interpretation which would support the data of Summerly, Ilderton & Gray (1978). However, the possibility of an increased incorporation into retinol derivatives, which are soluble in chloroform-methanol and which serve as intermediates in glycoprotein synthesis (De Luca, 1977) cannot be ruled out.

The surprising correlation between the incorporation of galactose into glycolipids and the percentage of 'large' cells in the keratinocyte preparation (Fig. 3) yields some insight into the site of galactose utilization in the epidermis. These cells, which possess a rigid keratinized structure but which still retain nuclei, are clearly derived from the upper layers of the stratum spinosum. Thus it would seem either that incorporation of galactose into lipid occurs chiefly in this region or that the incorporation of this particular sugar into glycoprotein and/or proteoglycans is limited to the basal and lower spinous layers.

By far the most interesting observation arising from the present work is the abnormality in the molecular weight distribution of fucose-containing glycopeptides, particularly since this confirms our previous report of a specific disturbance in the metabolism of fucose by psoriatic keratinocytes (Roelfzema *et al.*, 1981). The reduced C/B ratio in the untreated lesion indicates either that the fucosylated glycopeptides are more highly branched than normal or that there is an increased sialylation of glycopeptides with a normal degree of branching. A similar shift to higher molecular weight glycopeptides has been reported in cultured BHK cells following viral transformation (Takasaki, Ikehira & Kobata 1980); in this case it was established that the degree of branching was abnormally high.

During the past few years a number of changes have been reported to occur in clinically uninvolved psoriatic epidermis. In general, however, these alterations are in the same direction as those found in the lesion but are smaller in magnitude; thus it is difficult to exclude the possibility that such observations result from the presence of 'sub-clinical' lesions in the apparently healthy epidermis. It is therefore noteworthy that the abnormality which we have demonstrated in the uninvolved psoriatic epidermis is in the opposite sense to that found in the lesion, i.e. the molecular weight of fucosylated glycopeptides is reduced. Further work in this direction is in progress.

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The effects of topical and systemic glucocorticosteroids on DNA synthesis in different tissues of the hairless mouse

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SUMMARY

Two topical corticosteroids, clobetasol propionate and clobetasone butyrate, have been studied in hairless mice for their effects on DNA synthesis in the epidermis, thymus and spleen. Following topical application, both clobetasol propionate and clobetasone butyrate showed significant activity at the site of application throughout the range of concentrations tested (20 μl; 0·0001–0·1%; 20 ng to 20 μg). However, whereas 20 ng clobetasol propionate also elicited significant effects in the distal (untreated) epidermis and the thymus, more than 2 μg of clobetasone butyrate were required to produce similar effects in these tissues. This finding was supported by the results obtained following intravenous administration of equivalent doses (0·01 and 0·001%; 200 μl dose) of the same two steroids. Only clobetasol propionate showed significant activity in the epidermis and thymus. Clobetasone butyrate showed slight, non-significant effects in the epidermis at the highest concentration (200 μl; 0·01%), but not in the thymus or spleen. An unexpected finding was that the effects following intravenous injection were generally lower than those following topical application.

In conclusion, these results establish that (a) effects on DNA synthesis in the epidermis at a site distal to the application site are indicative of systemic activity from topically applied corticosteroids, (b) the thymus is especially sensitive to corticosteroids eliciting systemic effects and (c) an equivalent dose of a topical corticosteroid administered intravenously produces less inhibition of thymic and epidermal DNA synthesis than the same dose applied topically.

A hairless mouse model for evaluating the effects *in vivo* of drugs on epidermal cell mitosis (du Vivier *et al.*, 1976) and DNA synthesis (as measured by the incorporation of ^{3}H-thymidine) (du Vivier, Marshall & Brookes, 1978), has been described previously. The model has been used to determine the local and distal effects of topically applied butyrate esters of clobetasone and clobetasol and the propionate ester of clobetasol on the synthesis of epidermal DNA (Marshall & du Vivier, 1978). In these studies, distal (untreated) epidermis was chosen as the tissue in which to monitor systemic

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effects and the compounds were compared at one concentration only. The current studies were undertaken to (a) demonstrate the validity of this choice by examining the effects of these compounds in internal body tissues (thymus and spleen) as well as in the local and distal epidermis, (b) construct dose-response profiles for these two steroids in those tissues and (c) determine the differences, if any, between the effects of glucocorticosteroid esters on target organs following percutaneous and intravenous administration.

MATERIALS AND METHODS

Animals

Hairless mice (strain A2hr), random bred, 2–4 months old and of either sex were used. The animals were dosed in five groups of ten, each of four of the groups with a different concentration of the drug under investigation and the other group with the vehicle as control.

Drugs

(1) Clobetasol propionate (in Dermovate: Glaxo) (CP).
(2) Clobetasone butyrate (in Eumovate: Glaxo) (CB).

When applied topically, the steroids were tested at concentrations of 0·1%, 0·01%, 0·001% and 0·0001% w/v in an alcoholic solution (ethanol: propylene glycol, 70:30). For intravenous administration the steroids were tested at concentrations of 0·01% and 0·001% dissolved in an isotonic solution.

Procedures

Topical application. 20 μl of the test solution was applied, and spread over 3 cm^2 of the anterior dorsal skin (neck). The treated site was not occluded and the posterior half of the dorsal surface of the animal (back) was left untreated. At 23 h after the application all animals were given 25 μCi (methyl-^{3}H) thymidine, specific activity 5 Ci/mmol (The Radiochemical Centre, Amersham) subcutaneously in the right thigh and were killed by ether inhalation 1 h later. Samples of skin from the neck and back (with at least 2 cm of unsampled skin left between them to avoid inclusion of treated skin in the back sample) were removed. The back skin was dissected well away from the sites of drug application and ^{3}H-thymidine injection. The epidermis was separated from the dermis, in the skin samples, by heating at 56°C for 25 s. The thoracic and abdominal cavities were then exposed and the thymus and spleen removed. These latter samples were cleared of superficial blood and connective tissue. All samples were stored at -20°C until required for analysis. The DNA was extracted from the samples by column chromatography using hydroxylapatite, and its specific activity determined by UV-spectrophotometry and scintillation counting in the manner described previously (du Vivier *et al.*, 1978). DNA specific activity (^{3}H-thymidine incorporation) was initially expressed as mean disintegrations per minute (d.p.m.) per μg DNA ± standard error (s.e.) for the groups of animals. The significance of the effects, with respect to the control group, was determined by Student's t-test.

Intravenous injection. The test solution (200 μl) was injected into a lateral tail vein. At 17 h after this injection the animals received the (methyl-^{3}H) thymidine and were killed 1 h later. This earlier time (18 h) was selected because maximum effects following intravenous injection occur approximately 6 h earlier than those following topical application (unpublished results). The samples of neck epidermis were obtained as previously and the thymi and spleens were removed. These samples were processed as in the topical studies and the results expressed as mean d.p.m./μg DNA ± s.e.

RESULTS

The effects of clobetasol propionate (CP) and clobetasone butyrate (CB) in the different tissues 24 h after a single topical application are shown in Tables 1 and 2 respectively.

Both CP and CB showed significant local effects at the lowest concentration tested (0·0001%) although only CP showed a significant systemic effect (in distal epidermis and thymus) at this concentration. The degree of systemic effect of CP increased with concentration, until at 0·1% both local and systemic incorporation of ^{3}H-thymidine has been inhibited by greater than 90%. At this concentration however, CB was only just beginning to show significant systemic effects.

The effects of CP and CB on the different tissues 18 h after a single intravenous injection are shown in Table 3.

CP showed significant effects only at the highest concentration (0·01%) and then only in the epidermis and thymus. CB showed no significant effects in any of the tissues.

TABLE 1. The effects of clobetasol propionate (CP) on DNA synthesis at 24 h after topical application

Concentration of clobetasol propionate	DNA specific activity (d.p.m./μg DNA)							
	Local epidermis		Distal epidermis		Thymus		Spleen	
	Mean* ± s.e.	*P*	Mean* ± s.e.	*P*	Mean* ± s.e.	*P*	Mean* ± s.e.	*P*
Vehicle	115·1 ± 10·6		139·7 ± 14·5		40·7 ± 1·9		158·5 ± 10·5	
0·1%	8·0 ± 1·5	< 0·001	9·7 ± 0·8	< 0·001	2·4 ± 0·3	< 0·001	74·9 ± 7·4	< 0·001
0·01%	8·0 ± 1·5	< 0·001	40·1 ± 7·5	< 0·001	13·6 ± 2·5	< 0·001	88·8 ± 7·2	< 0·001
0·001%	22·8 ± 4·8	< 0·001	69·8 ± 8·0	< 0·001	25·7 ± 1·9	< 0·001	76·8 ± 5·1	< 0·001
0·0001%	51·9 ± 4·3	< 0·001	103·5 ± 7·0	< 0·05	34·1 ± 1·4	< 0·02	131·7 ± 10·7	N.S.

* n = 10.
N.S. = (not significant) *P* > 0·05.

TABLE 2. The effects of clobetasone butyrate (CB) on DNA synthesis at 24 h after topical application

Concentration of clobetasone butyrate	DNA specific activity (d.p.m./μg DNA)							
	Local epidermis		Distal epidermis		Thymus		Spleen	
	Mean* ± s.e.	*P*	Mean* ± s.e.	*P*	Mean* ± s.e.	*P*	Mean* ± s.e.	*P*
Vehicle	106·1 ± 5·9		93·9 ± 4·9		34·2 ± 2·4		140·6 ± 12·6	
0·01%	6·0 ± 1·0	< 0·001	38·5 ± 2·1	< 0·001	26·2 ± 1·9	< 0·02	135·1 ± 13·1	N.S.
0·01%	16·0 ± 2·2	< 0·001	73·0 ± 9·2	N.S.	27·9 ± 5·0	N.S.	145·7 ± 13·3	N.S.
0·001%	49·5 ± 6·0	< 0·001	88·6 ± 9·4	N.S.	37·6 ± 3·3	N.S.	123·5 ± 3·3	N.S.
0·0001%	62·6 ± 8·7	< 0·001	80·7 ± 4·8	N.S.	30·3 ± 2·3	N.S.	119·0 ± 15·3	N.S.

* n = 10.
N.S. = (not significant) *P* > 0·05.

TABLE 3. The effects of clobetasol propionate (CP) and clobetasone butyrate (CB) on DNA synthesis at 18 h after intravenous injection

Concentration of steroid	DNA specific activity (d.p.m./ug DNA)								
	Epidermis			Thymus			Spleen		
	Mean*	± s.e.	*P*	Mean*	± s.e.	*P*	Mean*	± s.e.	*P*
Vehicle	94.7	± 14.4	—	51·1	± 3·4	—	175·2	± 21·1	—
0·01% Clobetasol propionate	44·0	± 7·2	<0·01	18·9	± 5·0	<0·001	128·8	± 14·8	N.S.
0·001% Clobetasol propionate	78·3	± 8·5	N.S.	53·1	± 5·7	N.S.	131·9	± 13·6	N.S.
0·01% Clobetasone butyrate	74·5	± 6·2	N.S.	44·9	± 7·1	N.S.	131·5	± 14·9	N.S.
0·001% Clobetasone butyrate	89·6	± 6·2	N.S.	61·3	± 6·5	N.S.	183·3	± 9·5	N.S.

* n = 10.
N.S. = (not significant) $P > 0{\cdot}05$.

DISCUSSION

The results following topical CP and CB (Tables 1 and 2) confirm our earlier findings that CP is more potent than CB in its local effects and very much more potent in its systemic effects. The observation remains equally valid if systemic effects are measured in the thymus, the spleen, or the distal epidermis.

The difference in the activities of CP and CB are also seen when the two compounds are administered intravenously—only CP shows significant effects. Thus, the difference in systemic effects of the steroids following topical application is probably not due solely to a difference in rates of percutaneous absorption but to a difference in intrinsic molecular activities (Marshall & du Vivier, 1978).

It is interesting that a dose of CP applied topically elicits considerably greater systemic effects than the same dose administered intravenously. This is contrary to expectations. It implies that the peak blood concentration of the steroid ester is not the overriding factor in the production of systemic effects. Further study is required to ascertain if this anomaly is due to the difference in blood level profiles, following administration of the drug by the different routes. Topical application would produce a lower and more sustained profile of activity than that following intravenous injection.

In conclusion, we have shown that effects seen in the distal epidermis, following topical application of corticosteroids, represent systemic activity as measured in the thymus or spleen. We have also shown that the extent of the systemic effects of the corticosteroids in different tissues varies and that they are significantly influenced by the intrinsic activity of the drug molecule and the route by which it is administered.

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Allergic and toxic contact dermatitis: inflammatory cell subtypes in epicutaneous test reactions

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SUMMARY

Histochemical and immunohistochemical techniques were used to identify T lymphocytes, mononuclear phagocytes and plasma cells in situ from allergic and toxic epicutaneous test reactions. Intracellular α-naphthyl acetate esterase (ANAE), endogenous peroxidase and immunoglobulin were used as markers for inflammatory cells. In allergic contact dermatitis 76 ± 7% of all cells were ANAE-positive T lymphocytes, 13 ± 6% mononuclear phagocytes and 12 ± 6% ANAE-negative cells. In toxic skin lesions the corresponding values were 64 ± 20%, 18 ± 15% and 18 ± 6%, respectively. There were no statistically significant differences between the allergic and toxic skin reactions. The basic reaction type in allergic and toxic contact dermatitis seems to be similar, with possibly some qualitative and quantitative differences.

It is not usually possible to distinguish histologically between an allergic contact dermatitis and a primary irritant or toxic contact dermatitis (Lever & Schaumburg-Lever, 1975). However, the epicutaneous testing of sensitized individuals with the specific sensitizing substance and of nonsensitized individuals with a toxic substance results in different histological responses (Krawiec & Gaafar, 1975), though these differences could be of a primarily quantitative character (Nater & Hoedemaeker, 1976).

This study was undertaken to see if differences exist between allergic and primary irritant or toxic skin test responses as analyzed by histochemical and immunohistochemical methods for T lymphocytes, mononuclear phagocytes and plasma cells. An epicutaneous chamber test was used to elicit the test lesions (Pirilä, 1975).

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MATERIALS AND METHODS

Patients

The epicutaneous tests were applied on the patients' backs using the chamber test method (Pirilä, 1975). The chambers were removed after 24 h. In each patient one allergic and one toxic reaction was studied.

The allergic test reaction was evoked in five patients by 2·5% nickel sulphate and by 1·0% epoxy resin, 1·0% p-phenylenediamine and 1·0% diphenyl-p-phenylenediamine in one patient each. All these test substances were incorporated in petrolatum. The irritants were applied on the same day as the allergens. The irritant (toxic) reaction was provoked in seven cases with a 0·2–0·4% aqueous solution of mercury bichloride and in the remaining one case with 2·0% aqueous benzalkonium chloride. Hypersensitivity to mercury was excluded by a negative reaction to 0·1% mercury bichloride in petrolatum.

The test reactions were excised 1–3 days after application of the test substances, the allergic and toxic reactions at the same time. Care was taken to excise allergic and toxic reactions of similar strength from each of the patients.

Tissue processing

For the demonstration of α-naphthyl acetate esterase (ANAE) staining in T lymphocytes and mononuclear phagocytes the tissues were processed as described in detail elsewhere (Ranki *et al.*, 1980). Briefly, the tissues were fixed in Baker's formol calcium for 12 h at 4°C, washed in buffer, dehydrated in acetone and cleared in xylene, all at 4°C, before paraffin embedding.

For the immunoperoxidase demonstration of intracellular immunoglobulin the tissues were fixed in Bouin's fluid for 4–8 h (Reitamo & Reitamo, 1978). Subsequently, the tissues were dehydrated in absolute alcohol, cleared in xylene and embedded in paraffin according to the standard histological technique.

Histochemical demonstration of ANAE

Tissue specimens deparaffinized in xylene and then in acetone (10 min each at 4°C) were incubated in a medium consisting of 40 ml of 0·0067 mol/l phosphate buffer, pH 5·3, 2·4 ml of hexazotized pararosaniline, and 10 mg of α-naphthyl acetate (Sigma Pharmaceuticals, St Louis, Mo., U.S.A.) in 0·4 ml of acetone. The mixture was adjusted to pH 5·8 using 1 mol/l NaOH. A 3–4 h incubation time was used. Counterstaining was performed in a 1% aqueous solution of toluidine blue, the sections were dehydrated in methanol and then acetone and cleared in xylene, 10 min each, and mounted (Ranki *et al.*, 1980).

Histochemical demonstration of endogenous peroxidase

Tissue sections were incubated in darkness for 30 min with 3′,3′diaminobenzidine (Sigma Chemical Co, St Louis, Mo., U.S.A.) and H_2O_2 (DAB 75 mg/100 ml phosphate-buffered saline, pH 7·2 and 0·003% H_2O_2) as described by Graham & Karnovsky (1966). Toluidine blue was used for counterstaining.

Immunohistochemical demonstration of intracellular immunoglobulin

Tissue sections were deparaffinized in xylene and rehydrated through graded alcohols and then washed in buffer. The intrinsic peroxidase activity of granulocytes, mononuclear phagocytes and erythrocytes was destroyed by pretreating tissue sections in methanol-H_2O_2 (Streefkerk, 1972). The immunoglobulins were demonstrated with the peroxidase-anti-peroxidase method

(Sternberger *et al.*, 1972) as described in detail elsewhere (Bergroth *et al.*, 1980). The tissue sections were treated with: (1) normal swine serum (dilution 1:5), (2) rabbit anti-human immunoglobulin, isotypes IgG, IgM, IgA, IgD, IgE, κ or λ light chains (1:600, Dakopatts, Copenhagen, Denmark), washed with PBS and treated thereafter with (3) swine anti-rabbit IgG, heavy and light chain specific (1:200, Dakopatts), and (4) horseradish peroxidase rabbit anti-horseradish peroxidase complexes (PAP) (1:200, Dakopatts). The sections were finally stained by the method of Graham and Karnovsky and counterstained with haematoxylin. Plasma cells were also identified with Unna-Pappenheim staining.

To test the specificity of the immunohistochemical reaction, the following controls were performed: (1) instead of the primary anti-Ig the sections were treated with normal rabbit serum; (2) the primary antisera were absorbed with the respective purified antigen.

Quantification of inflammatory cells in skin sections

Inflammatory cell subclasses were counted from the stained slides (6 μm tissue sections) in the upper dermis using an ocular counting square (20 squares × 20 squares) and an oil-immersion objective (× 1000 magnification). At least 200 inflammatory cells were counted in each test reaction. The investigator counting the cells did not know the source (allergic or toxic reaction) of the sample.

RESULTS

ANAE, peroxidase and immunoperoxidase methods enabled unequivocal analysis of the relative proportions of the inflammatory cell subtypes in relation to each other and with respect to the tissue structures. The T lymphocytes were identified in ANAE staining by one or more

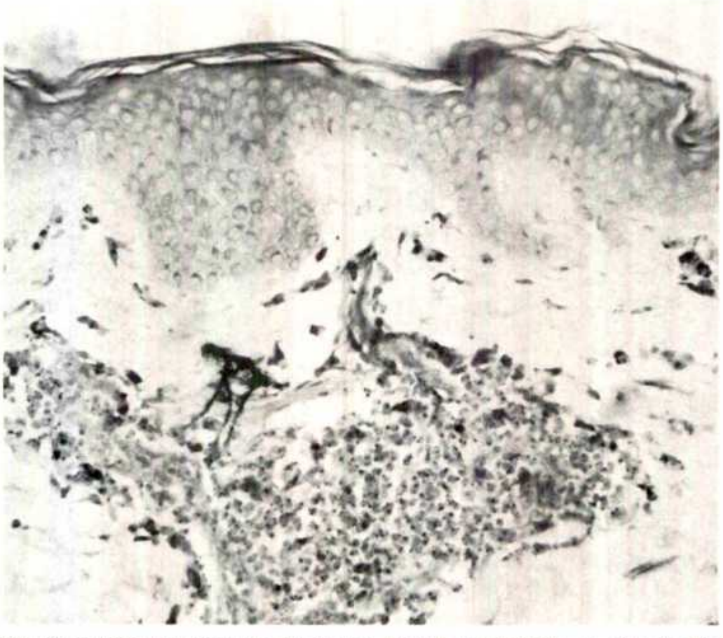

FIGURE 1. Cells of perivascular infiltrate displaying ANAE reactivity in an allergic skin reaction. T lymphocytes showing one to several distinct dots in the cytoplasm and mononuclear phagocytes showing diffuse cytoplasmic ANAE reaction (× 200).

TABLE 1. Inflammatory cell infiltrate in allergic and toxic skin lesions

	Allergic skin lesion				Toxic skin lesion			
	Extent of infiltrate*/ cells per 5000 μm²	Inflammatory cells			Extent of infiltrate*/ cells per 5000 μm²	Inflammatory cells		
Patient		T pattern†	M pattern‡	Non-T Non-M§		T pattern†	M pattern‡	Non-T Non-M§
U.T.	+ + +/121	73%	17%	10%	+ +/110	80%	2%	18%
I.T.	+/88	83%	4%	13%	ND	ND	ND	ND
U.K.	+ + +/210	85%	7%	8%	+/125	50%	33%	17%
P.J.	+ + +/202	70%	22%	8%	+/115	46%	33%	21%
R.J.	+ +/186	66%	11%	23%	+/109	40%	30%	30%
E.P.	+ +/84	74%	18%	8%	+ +/132	84%	2%	14%
L.S.	+ + +/124	80%	14%	6%	+ + +/197	86%	3%	11%
J.L.	+ +/174	74%	10%	16%	+/84	56%	27%	17%
	Mean ± SD	76 ± 7	13 ± 6	12 ± 6		63 ± 20	18 ± 15	18 ± 6

* Graded from − to + + +.
† ANAE-positive T lymphocytes.
‡ ANAE-positive mononuclear phagocytes.
§ ANAE-negative inflammatory cells.
ND = not done.

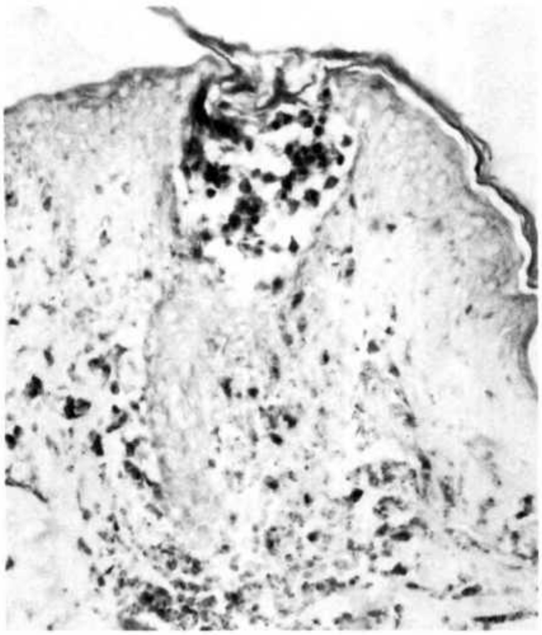

FIGURE 2. An allergic skin reaction with a spongiotic intra-epidermal vesicle containing mainly mononuclear phagocytes staining diffusely with ANAE. The infiltrate in the upper dermis consists mainly of T lymphocytes (× 200).

distinct reaction products in the cell cytoplasm whereas the mononuclear phagocytes displayed a diffuse ANAE activity (T and M pattern; Fig. 1).

There was usually more of a T lymphocyte-rich dermal infiltrate in allergic skin reactions (Fig. 1) than in toxic skin reactions (Table 1). Allergic skin reactions were characterized by intra-epidermal spongiotic vesicles, whereas toxic skin reactions were characterized by more epidermal damage and subepidermal blister formation. The intra-epidermal spongiotic vesicles (Fig. 2) and subepidermal blisters (Fig. 3) contained ANAE positive mononuclear phagocytes, and sometimes even granulocytes. In most allergic skin reactions there were more mononuclear phagocytes near the epidermis than in the deeper perivascular dermal infiltrates. There was a good correlation between the number of ANAE and peroxidase-positive mononuclear phagocytes in consecutive sections in individual patients.

In allergic test reactions the inflammatory infiltrates in the upper dermis contained 76 ± 7% T lymphocytes from all cells, 13 ± 6% mononuclear phagocytes and 12 ± 6% ANAE-negative round cells, mostly lymphocytes (Table 1). In toxic test reactions the corresponding figures were 63 ± 20%, 18 ± 15% and 18 ± 6%. When these figures were compared using the t-test, there were no significant differences between the allergic and toxic skin lesions. Unna-Pappenheim positive cells were infrequent (less than 1% of all inflammatory cells) in both reaction types: the relative infrequency of the plasma cells was confirmed by immunoperoxidase staining of intracellular immunoglobulin.

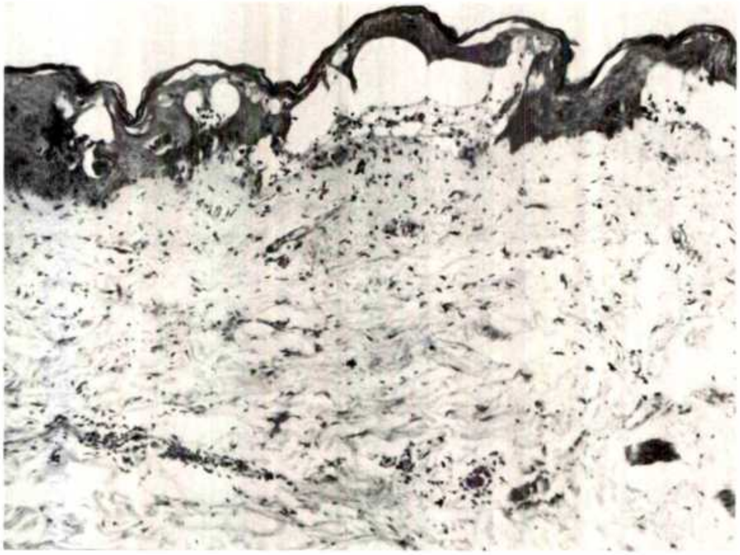

FIGURE 3. A toxic skin reaction with a subepidermal blister formation. Most of the inflammatory cells in this blister are mononuclear phagocytes staining diffusely with ANAE. In spite of the strong epidermal reaction there is little infiltrate in the dermis (× 70).

DISCUSSION

Identification of inflammatory cells in tissue sections using surface markers (antigenic structures and receptors) is difficult. Surface markers are not as exposed in tissue sections as they are in cell suspensions, but are too exposed to be preserved during tissue processings up to paraffin embedding. Immunoglobulin, complement and any immune complexes present in tissue sections can interact with inflammatory cells, indicator cells or indicator antibodies. Morphological characterization of individual cells underneath the indicator cells or fluorescent antibodies is impossible. For this reason intracellular markers were employed for inflammatory cells in the present work. ANAE is present in more than 80% of lymphocytes forming rosettes with sheep red blood cells and in less than 10% of lymphocytes bearing surface immunoglobulin (Ranki, Tötterman & Häyry, 1976). The first paraffin technique for human T lymphocytes in tissue section was recently published (Ranki *et al.*, 1980). Non-specific esterases present in mononuclear phagocytes also split α-naphthyl acetate, but the staining patterns are different (T lymphocyte-cytoplasmic dot(s), mononuclear phagocytes—diffuse cytoplasmic staining). In addition, *in situ* characterization of mononuclear phagocytes was done with endogenous peroxidase, which is also present in granulocytes. Plasma cells were demonstrated using the histochemical Unna-Pappenheim technique and different intracellular immunoglobulin isotypes were characterized with an immunoperoxidase technique which allows paraffin embedding and counterstaining (Reitamo & Reitamo, 1978). The present study shows that the ANAE-positive T lymphocyte is the dominant cell in both allergic and toxic contact test reactions. The proportion of T-cells found in the present study is in good accordance with earlier findings by Schmitt, Alario & Thivolet (1979), who demonstrated T lymphocytes in allergic contact dermatitis with a specific heteroantiserum. Cormane (1974) showed that most lymphocytes *in situ* in allergic contact dermatitis were surface immunoglobulin bearing, but it was not possible to demonstrate whether the surface immunoglobulin was endogenously synthesized or exogenously acquired.

Using both ANAE and peroxidase as a marker for mononuclear phagocytes, we were able to demonstrate that mononuclear phagocytes were the dominant cell in the subepidermal and intraepidermal blisters. This is in accordance with earlier findings by Bandmann (1967). However, the use of the ANAE method to demonstrate T lymphocytes and mononuclear phagocytes in the same slide clearly demonstrated that in the perivascular dermal infiltrates the T lymphocyte was the dominant inflammatory cell. This was confirmed with the peroxidase technique. It is difficult to explain the differences between the present results and those of Bandmann (1967), who used endogenous peroxidase as a marker for mononuclear phagocytes and also showed this cell subtype to be the dominant cell in the perivascular infiltrates.

The non-T, non-M cells were not morphologically polymorphonuclear granulocytes or plasma cells, neither did they contain intracellular immunoglobulin. They are most probably B lymphocytes (Ranki *et al.*, 1976), though they could also be null cells (Higgy, Burns & Hayhoe, 1977), suppressor T lymphocytes (Grossi *et al.*, 1978) or activated T lymphocytes (Tötterman, Ranki & Häyry, 1977).

There were fewer mononuclear phagocytes and non-T, non-M cells and more T lymphocytes in the allergic skin lesions than in the toxic skin lesions. However, these differences were not statistically significant. It is difficult to exclude the possibility that these slight differences could be more dependent on the reaction intensity or developmental phase of the reaction than on the initiating cause.

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Monilethrix: an electron microscopic and electron histochemical study

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SUMMARY

We have carried out electron microscopic and electron histochemical investigations on affected hair follicles and hair shafts to elucidate further the structural abnormality in monilethrix. The structural abnormalities manifest in the cortex and cuticle of the hair shaft could be traced to the locality of the 'zone of keratinization' of the follicle. Abnormal orientation of the cortical cell proteins, folding of the cuticle cell membrane and varying amounts of endocuticular material were all observed in the region prior to full keratinization. We observed structural abnormalities within all the component layers of the inner root sheath adjacent to the faults within the hair shaft, indicating a possible asynchrony within the monilethrix follicle.

Monilethrix is a genetic disease characterized by beading of the hair shaft resulting from the alternation of the wider nodes and the narrower internodes. The unusual geometric form of this hair is such that simple procedures such as brushing, combing, washing and general handling can be expected to cause far more damage to the shaft than in normal hair. Indeed, individuals with this abnormality usually have quite short hair as a consequence of premature fracture of the hair at the narrower internodal sections. Coupled with this, the hair surface is seen by scanning electron microscopy to be more severely damaged than the corresponding part of normal hair (Dawber & Comaish, 1970; Dawber, 1979); whereas the cuticular surface in the nodal sections tends to resemble that of normal hair, the surface in the internodal sections is curiously fluted indicative at least in these regions of the hair shaft, of an intrinsic cuticular and/or cortical structural abnormality. In this study we have carried out electron microscopic and electron histochemical investigations on affected hair follicles and hair shafts to elucidate further the structural abnormality in monilethrix.

MATERIALS AND METHODS

Affected hair shafts from four patients were embedded in Spurr's low viscosity resin (Spurr, 1969). Transverse sections approximately 50 nm thick were cut at mid-nodal and mid-interno-

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dal sites using a diamond knife on an LKB Mk III Ultratome. The sections were stained with: (1) uranyl acetate and lead citrate, a general morphological staining technique (Reynolds, 1963); (2) silver methenamine (Swift, 1968) as a cystine-specific stain to show relative amounts of cystine amongst the various sub-components of the hair shaft.

Scalp biopsy tissue was immersed in half strength Karnovsky's fixative (Karnovsky, 1965) and then dissected into blocks containing approximately three follicles. These were then placed in fresh fixative for 2 days, then post-fixed with 2% osmium tetroxide, followed by dehydration in graded ethanol and propylene oxide (overnight); the embedding material used was Spurr's low viscosity resin. Although long exposure to epoxy propane caused some leaching of the membranes of the dermal tissue, previous experience had shown it to be essential in order to remove all the alcohol from the subcutaneous fat. A low viscosity resin was required to facilitate complete infiltration into the follicular canal.

Semi-thin (1–2 μm) and ultra-thin (60 nm) longitudinal sections of anagen follicles were cut on an LKB Mk III Ultratome and stained as follows: semi-thin—(1) toluidine blue, (2) Humphrey's, methylene blue, azure II, basic fuchsin (Humphrey & Pittman, 1974); ultra-thin—(1) uranyl acetate and lead citrate (Reynolds).

All ultra-thin sections of both the hair shaft and the follicle were viewed on an Elmiskop 101 D electron microscope.

RESULTS

The hair shafts were studied at two main sites, mid-node and mid-internode; each of these sites were studied at both proximal and distal locations on the hair shaft.

Cuticle

Node. There was a marked decrease in the number of cuticle cell layers in comparison with adjacent internodes at both proximal and distal locations on the hair shaft. Apart from this the internal structure of the cuticle resembled that of normal hair, showing in transverse section a concentric arrangement of smoothly-bounded cuticle cells each containing the normal complement and distribution of A-layer, exocuticle and endocuticle sub-components (Fig. 1).

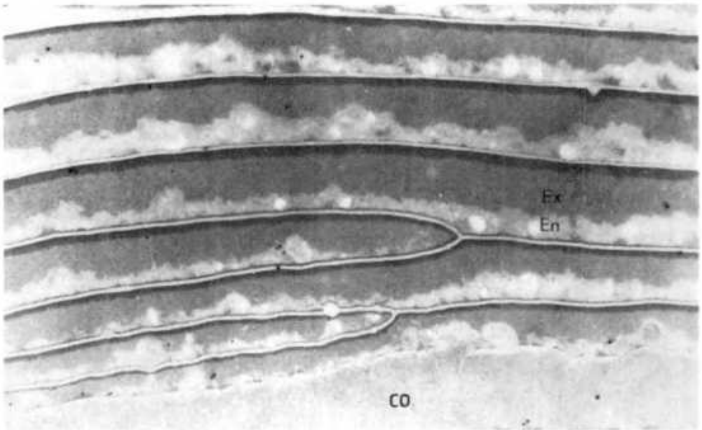

FIGURE 1. Transverse section of the cuticle at the node. The cuticle cell membranes are in regular concentric arrangement with normal endocuticle (En) and exocuticle (Ex). Cortex (Co). Stain: silver methenamine.

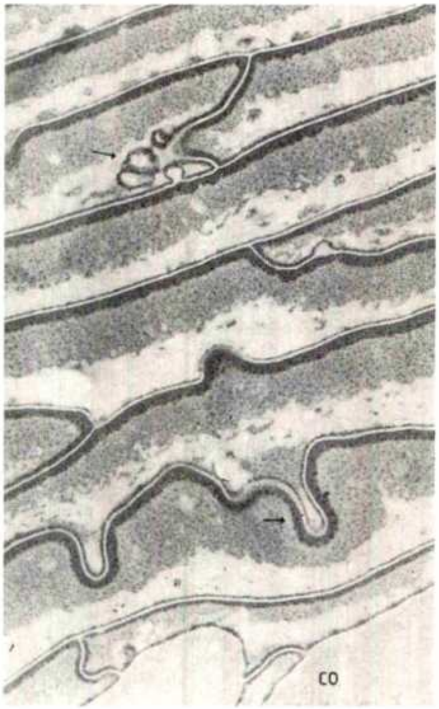

FIGURE 2. Transverse section of the cuticle of an internode showing folding of the cuticle cell membrane (arrow). Cortex (Co). Stain: silver methenamine.

Internode. Externally the cuticle was seen to degenerate progressively from the proximal to the distal end of the hair shaft. Longitudinal ridging and cuticular cell breakage increased distally, the terminal internodes being totally devoid of cuticular material leaving the cortex exposed. All the fibres examined showed terminal 'brush breaks' similar to trichorrhexis nodosa.

In transverse section the membranes of the cuticle cells showed marked irregular folding, the folds affecting the whole lamellar structure of the involved cuticle cells (Fig. 2). It appeared that those cuticle cells immediately adjacent to the cortex were most affected. Whereas within normal hair the boundary between the cuticle and cortex is usually of smooth contour, in monilethrix internodal regions there is considerable interdigitation between the cuticle and cortex (Fig. 3). In several cases discrete islands of cuticular material were encountered within the cortex a short distance from the cuticle/cortex boundary and vice versa.

Despite the gross irregularity of the cuticle in the internodal regions and the considerable variation in the local thickness of each cuticle cell, the A-layer sub-component was still found to be of regular thickness (about 120 nm) contouring the cell boundary on the outer-facing aspect of each cuticle cell. In addition to this each cell was also sub-divided into exocuticular and endocuticular components respectively in the outer-facing and inner-facing aspects of each cell, albeit with somewhat irregular thickness in different parts of each cell. The boundary between the exocuticle and endocuticle was not as clearly contoured as in normal hair and there was some evidence for mixing of the two components about the interface (i.e. regions of the endocuticle within the exocuticle and vice versa). In some cases there was evidence of nuclear remnants

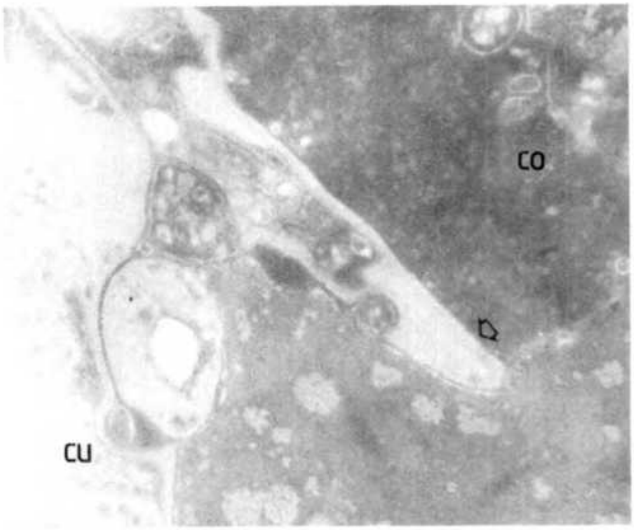

FIGURE 3. Shows cuticular inter-digitations (arrow) with the cortex (Co). Stain: uranyl acetate/lead citrate.

located within the endocuticle. In normal hair cuticle, the nucleus is dispersed within the endocuticle so that it is not morphologically evident with the type of stains used in the present work.

There was an increased incidence of pigment granules within the cuticle cells of the internodes at all levels throughout the length of the hair and throughout each of the cell layers.

Cortex

Node and internode. Though apparently normal cortical cells were encountered, most cells, particularly at the internodes were abnormal.

Many cells showed a lack of macrofibrillar organization (Fig. 4). In the normal cortical cell the macrofibrils appeared as well-ordered units in close proximity, whereas in the affected cortex they showed poor substructure, often with electron-opaque material interceding between the macrofibrillar units. The intermicrofibrillar distances within the macrofibrils varied greatly (Fig. 4). The normal short-range hexagonal arrangement of microfibrils was rarely seen. Pockets of cystine-positive material were seen in many cortical cells, these pockets lacking microfibrillar units (Figs 4 and 5). Figure 5 shows the great contrast between apparently normal and abnormal cells. The cell to the left shows numerous macrofibrillar structures in close proximity with each other. Numerous microfibrils can be seen within each macrofibril with regular inter-microfibrillar distances. Some of the macrofibrils in this particular cell exhibit an apparent hexagonal array of microfibrillar sub-units. In contrast the adjacent cell shows a number of abnormalities. The close association of defined macrofibrillar units is not evident. There is a wide range in the intermicrofibrillar distances throughout this portion of the cell and indeed many pockets of cystine positive staining material can be seen with no microfibril present. Reorientation of areas of individual cortical cells were seen throughout the transverse sections of the hair shafts and can be seen in this micrograph, the cortical cell material is in part orientated away from the longitudinal axis of the hair shaft.

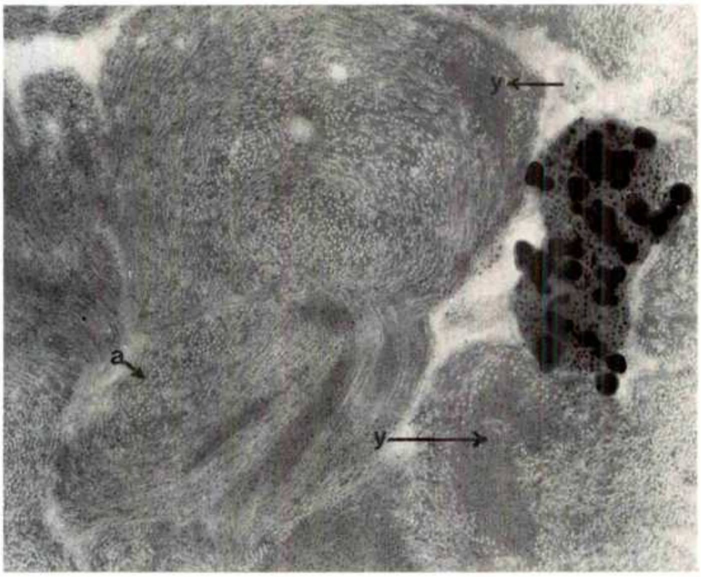

FIGURE 4. Transverse section of the cortex showing a wide variety of intermicrofibrillar distances (a), the microfibrils appearing as white dots. Cystine positive pockets of the material without microfibrils are evident (y). Stain: silver methenamine.

All the cuticle membranes and included pigment granules appeared to be normal in the sections studied.

The hair follicle

Inner root sheath. Henle's layer, normally a cylindrical single cellular layer of cells laid end to end, showed marked 'kinking' at one or two sites within the length of the follicles. These kinks were evident only in the fully keratinized zone of this layer (Fig. 6). Overlapping and bending of the cells as well as indenting of their usually regular membranes were also seen (Fig. 7).

Huxley's layer. No obvious abnormalities were noted in this layer though disorientation of the cells was obvious at the sites of 'kinking' in the Henle layer. Keratinization appeared to be normal in all the follicles studied.

Internal root sheath cuticle. Whereas in the normal hair follicle there is a well-ordered single layer of parallelogram-shaped inner root sheath cuticle cells that closely interlock with the hair shaft cuticle (Fig. 8), in monilethrix the cells varied considerably in shape (Fig. 8) with their nuclei also tending to adopt the irregular shape of each cell that contained them. As in the Henle layer these abnormalities were only evident in those cells that were either completely hardened or nearing completion of the process. Those cells in the region between the lower bulb and the zone of keratinization appeared normal. The junctions between the internal root sheath cuticle and the hair shaft cuticle were not of the simple tooth-like pattern seen normally, but often showed complex interlocking convolutions (Fig. 10).

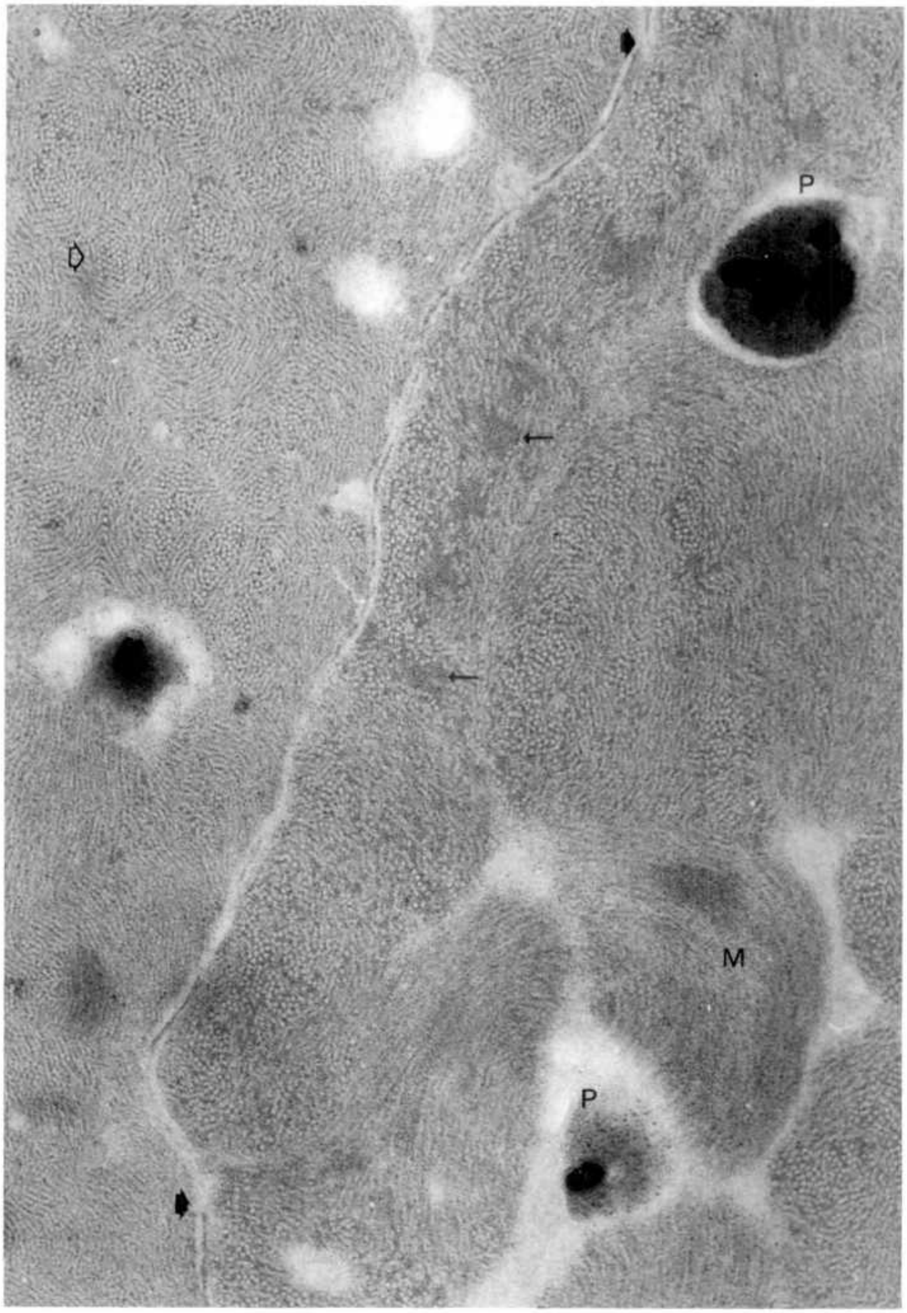

FIGURE 5. Transverse section of the cortex showing portions of the two adjacent cells. The cell to the left appears to be normal showing numerous macrofibrils with evidence of hexagonal substructure (⇒). The cell to the right shows pocketing of cystine positive material (→) and varied intermicrofibrillar distances. A macrofibrillar arrangement away from the longitudinal axis of the hair shaft can be seen (M). Pigment granules (P); Cell membranes (➡). Stain: Silver methenamine.

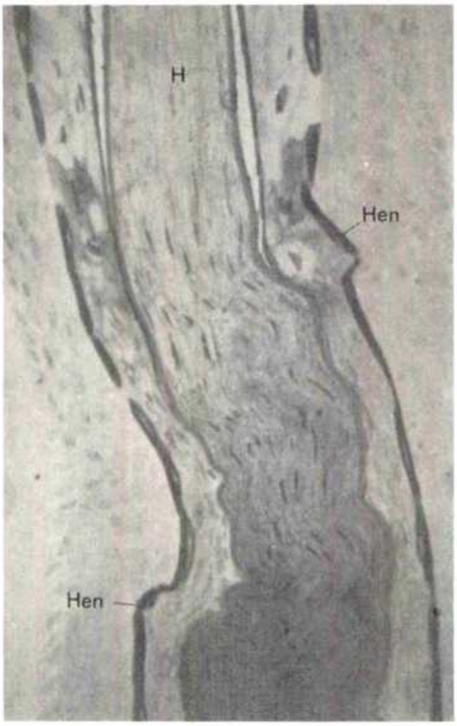

FIGURE 6. Longitudinal section of an affected follicle showing kinking of the inner root sheath, particularly Henle's layer (Hen). Hair shaft (H). Stain: Humphrey's.

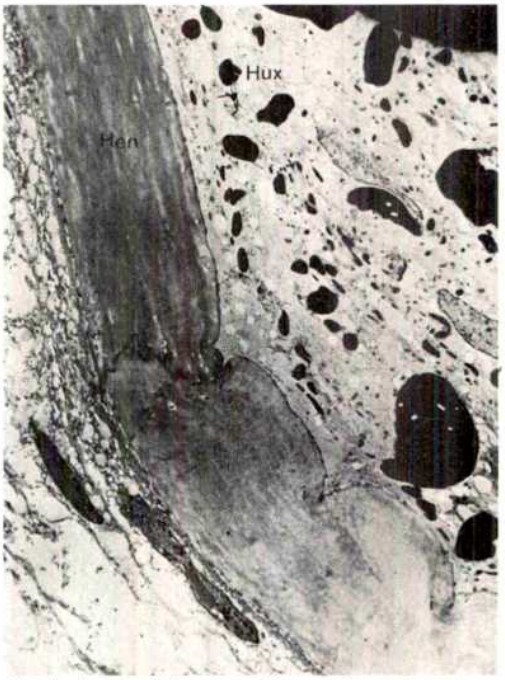

FIGURE 7. The inner root sheath showing indenting of the membrane (i) of a Henle cell. Henle layer (Hen). Huxley's layer (Hux). Stain: uranyl acetate/lead citrate.

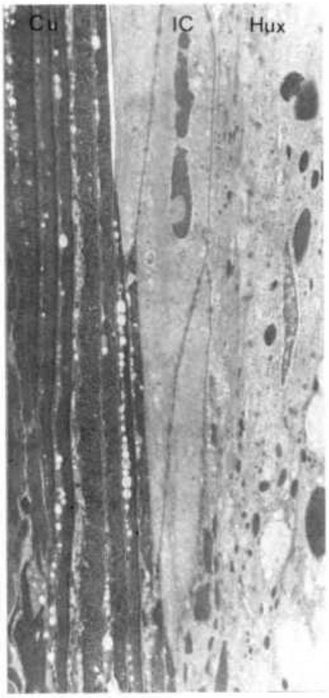

FIGURE 8. Longitudinal section from a normal follicle showing the close relationship of the inner root sheath cuticle (IC) with the hair shaft cuticle (Cu). Huxley's layer (Hux). Stain: uranyl acetate/lead citrate.

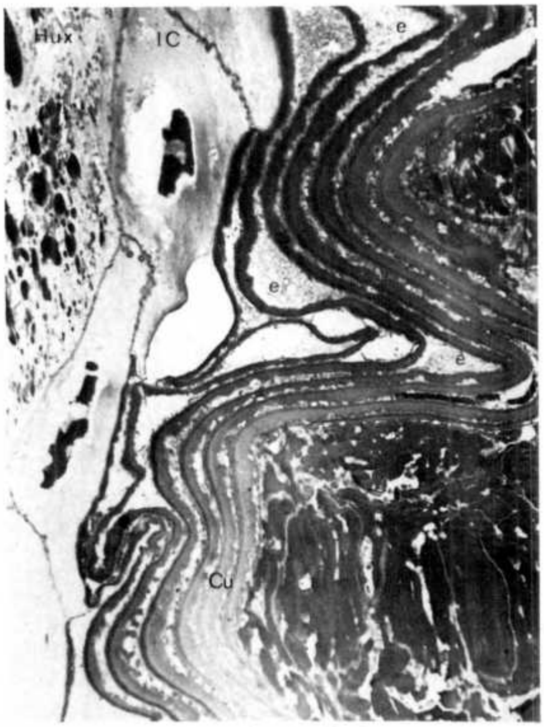

FIGURE 9. Longitudinal section from an affected follicle. The inner root sheath cuticle cells (IC) exhibit a wide variety of abnormal shapes other than the characteristic parallelogram shape. Note the gross folding of the hair shaft cuticle (Cu) and an increase in the endocuticle area (e). Stain: uranyl acetate/lead citrate.

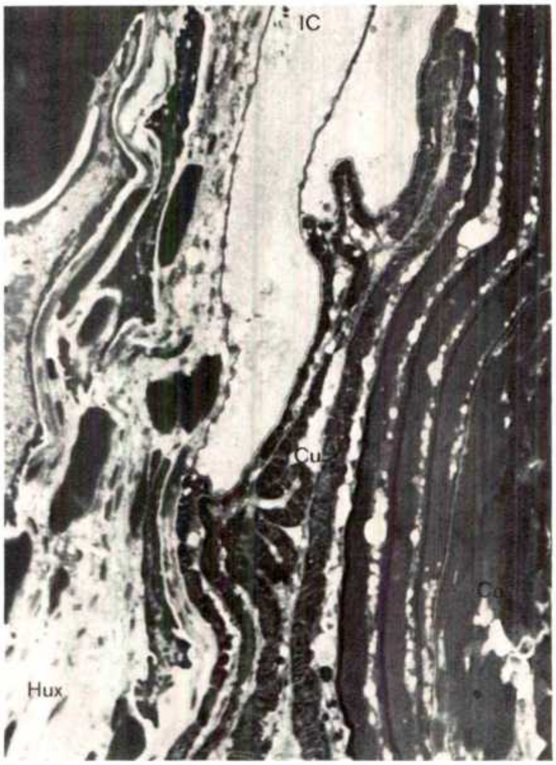

FIGURE 10. Longitudinal section from an affected follicle. The junction between the inner root sheath cuticle (IC) and the hair shaft cuticle (Cu) show highly involved folds, differing from the simple junctions seen in Fig. 8. Huxley's layer (Hux). Cortex (Co). Stain: uranyl acetate/lead citrate.

The hair shaft

Cuticle. This structure showed marked foldings about its longitudinal axis throughout all the stages of keratinization (Figs 10 and 11). The folds appeared to be of two main types occurring singly or together; those which involved the membrane of each individual cell (Fig. 10), and those that involved the cuticle as a whole unit (Fig. 9), the latter being the more severe and convoluting deep into the hair cortex (Fig 11). In both types of folding, the endocuticle appeared to occupy a greater than normal area of the respective cell. There was no obvious change in the width of the exocuticle or A-layer. In one of the affected follicles studied the presumptive cuticle cells, normally orientated with the developing free scale edge directed obliquely away from the cortex and in the direction of the hair tip, were found to be in the reverse position with the free scale edge directed obliquely away from the cortex and towards the base of the follicle.

Cortex. The cortex showed obvious disorientation manifested by the microfibrillar components not being orientated parallel to the longitudinal axis of the hair shaft as is a characteristic feature of normal hair. This abnormality was only seen at the sites of folding of the hair shaft cuticle, the protein fibrils curving around the involuting cuticle cells (Fig. 12). The sequence of events of keratinization did not appear to be affected.

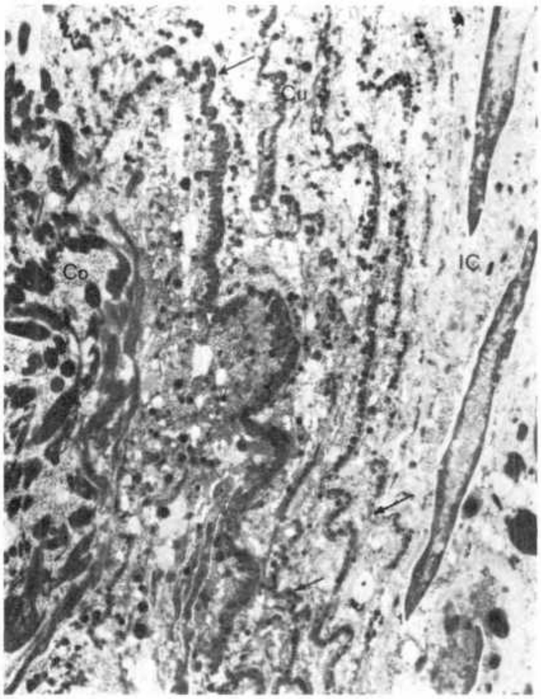

FIGURE 11. Longitudinal folding of cuticular membranes of the hair shaft in the early stages of keratinization. Cuticle (Cu). Cortex (Co). Internal root sheath cuticle (IC). Stain: uranyl acetate/lead citrate.

DISCUSSION

The characteristic weathering pattern in monilethrix is, we believe, due to two main factors.

(1) The gross geometry of the hair fibre which would account for the increased rate of distal weathering (cuticle cell loss) seen at the nodes as they are in relief with respect to the internodes, and a probable predilection for flexion of the fibre at the internodes due to the reduced bore, resulting in excessive trauma and eventual fracture.

(2) Intrinsic abnormalities of the internal structure of the hair shaft.

This study has shown that the cross sectional ultrastructure of the nodes is comparable with that of normal hair. (Swift, 1977, 1979). No evidence was found to suggest intrinsic abnormalities of the nodes. However, a number of abnormalities of both cuticle and cortex were observed at the internodes.

The cortex is the main structural element of the hair shaft, responsible for its great strength and flexibility. This study has shown a number of ultrastructural abnormalities within the cortex affecting the highly ordered relationship between the low-sulphur microfibrils and the high-sulphur ground matrix of the individual cells that is a characteristic feature of normal hair. These included a wide range of intermicrofibrillar distances, areas of cystine-positive material that lacked microfibrillar units, poor delineation at the macrofibrillar units and areas of cystine-positive material which did not show a definite ultrastructure. These abnormalities were typical of the many internodes studied. The specific cause of the above changes is not known but could be accounted for by one or a combination of the following:

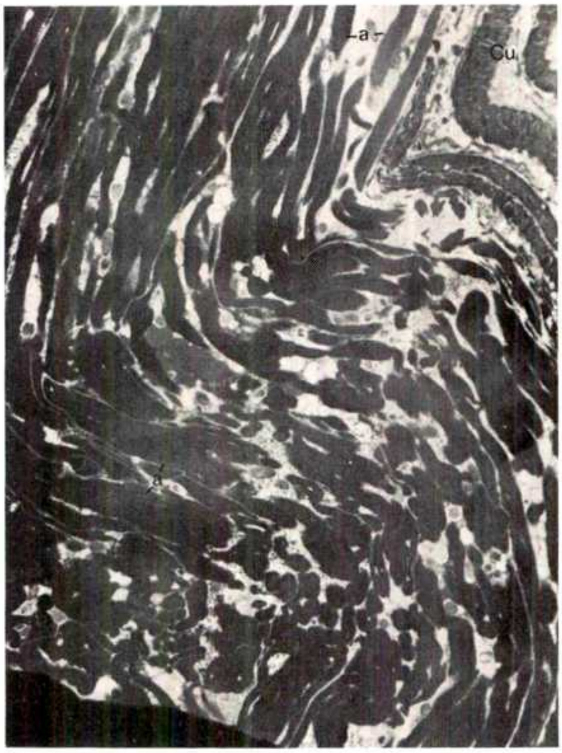

FIGURE 12. Longitudinal section of the hair shaft cortex showing re-orientation of the fibrillar proteins (a) away from the longitudinal axis of the hair shaft at the site of cuticular indentation (Cu). Stain: uranyl acetate/lead citrate.

(1) Insufficient cortical proteins produced to occupy the space available within the confines of the hair shaft cuticle, indicating that too few amino-acids are taken up by the active follicle.

(2)Insufficient amino-acids to form the low sulphur microfibrillar units or high sulphur matrix proteins or both.

(3)Abnormalities at the level of genetic information transfer resulting in malformation of the microfibril/matrix composite.

The transverse folds in the cuticle cell membrane observed at the internodes have been described, though not fully explained, in nasal hairs and in the coat hairs of the Australian swamp wallaby (Piper, 1967). It is possible that these folds, combined with the abnormal increase in pigment granule inclusion to the cuticle and the interdigitation of those cuticle cells adjacent to the cortex, are evidence of phagocytic activity of the cuticle cells prior to keratinization, a function normally confined to the keratinocytes of the cortex which phagocytose melanosomes from melanocytes. The limited thickness of the sections does not yield information about the structural elements above and below that section and hence the 'discrete islands' of cuticular material observed may simply be cross sections of folded cuticular processes, or indeed they may be separate entities.

The longitudinal folds observed in the cuticle would seem to indicate active compression of the cells. It is interesting that similar distortion affected the internal root sheath cuticle at the same follicular level. In the normal follicle the internal root sheath cuticle, which keratinizes first, and the developing hair shaft, move through the follicle at the same rate; however, the results appear to indicate asynchrony of movement, possibly of the hair shaft. It is interesting to note in this respect that in his studies of chemical shearing in wool, in which oral administration of the amino acid mimosine causes constrictions in the wool shaft, Chapman (personal communication) also found that the wool fibre keratinizes before the onset of hardening of the inner root sheath. It is likely that disruption would occur if the hair shaft cuticle were to be pushed past the opposing internal root sheath cuticle.

The regular beading of the hair shaft may be due to one of two factors or a combination of both:

(1) Intermittent activity of the basal cells producing alternate normal and reduced amounts of cortical protein units may cause a reduction in shaft diameter with complementary restriction of the cuticle diameter resulting in cuticle membrane folding.

(2) Intermittent activity of the internal root sheath, in particular the multicellular Huxley layer. One could suppose regular increases in the thickness of the internal root sheath which in the normal follicle acts as a mould for the hardening hair shaft. Although kinking of the internal root sheath was seen with complementary disorganization of the hair shaft, no regular changes in this structure were observed. This in itself or combined with asynchronous development of the shaft and internal root sheath as suggested would account for both the longitudinal and transverse disorganization of the hair shaft.

In the formation of the new hair shafts during the anagen phase of the hair growth cycle, the bore at the tip is less than that of the final hair. It may be supposed that via a feedback mechanism between the internal root sheath and the basal cells, the follicle is periodically thrown into a state of pseudo-catagen in the hair growth cycle, and that instead of the follicle achieving the telogen phase, the anagen phase is restored to give the full diameter fibre. This may in turn account for the periodic beading of the monilethrix hair shaft.

It is difficult to relate the ultrastructural abnormalities of the hair shaft and the gross structural changes of the follicle seen in this study to the regular beaded hairs that results in monilethrix. To elucidate further the structural changes it would be necessary to work on a far larger sample and to use radiolabelled amino acids in order to study their incorporation into the follicle and in particular, the internal root sheath. However, this would necessitate serial biopsies. Unfortunately, there is no comparable disease in laboratory animals to allow the use of an animal model.

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Cholinergic urticaria with associated angio-oedema

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SUMMARY

Three patients are described who developed angio-oedema and urticaria after a hot bath challenge and physical exercise. In one patient this was accompanied by a fall in peak expiratory flow rate. Blood levels of smooth muscle biological activity, measured using a bio-assay, increased in all patients after experimental challenge. The association of angio-oedema with cholinergic urticaria is emphasized and possible mechanisms discussed.

Cholinergic urticaria is a common and sometimes a disabling form of urticaria. It is characterized by short lived, small weals on an erythematous base evoked by exercise, passive overheating or emotional stimuli (Morgan, 1953). Commens & Greaves (1978) have reviewed the methods used to establish the diagnosis. The cutaneous response is regulated by cholinergic sympathetic fibres (Herxheimer, 1956), and seems to be associated with stimulation of the neuroglandular junction of the eccrine sweat gland. Wealing does not depend on sweating itself or on the composition of the sweat secretion (Illig & Heinicke, 1967a,b). Acetylcholine released from sudomotor nerve endings is not thought to cause mast cell degranulation directly; the delay between the release of acetylcholine and the appearance of weals suggests the presence of some intermediate link (Herxheimer, 1956). This may involve an IgE immunoglobulin in some cases as passive transfer by a stable reagin-like substance has been demonstrated (Illig & Heinicke, 1967c). (The nature of the antigen causing IgE stimulated mast cell degranulation is uncertain.) Recent work by Eftekhari *et al.* (1980) suggests that pharmacologically active degradation products of protein may be involved in the pathogenesis of cholinergic urticaria.

Various mediators of this response have been proposed (Soter & Wasserman, 1979). Plasma histamine levels rise after experimental challenge (Kaplan *et al.*, 1975; Matthews & Pan, 1970)

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suggesting that mast cell degranulation products are involved. Associated symptoms include abdominal cramps, diarrhoea, salivation (Paver, 1970) and wheezing (Soter *et al.*, 1980).

Classical angio-oedema presents as localized oedema extending beyond the dermis into the submucosa and subcutaneous tissues. Lesions commonly occur on the eyelids, lips and tongue (Champion *et al.*, 1969) but may appear anywhere. While angio-oedema has been reported in cholinergic urticaria (Hopkins, Kesten & Hazel, 1938), recent reviews do not comment upon this aspect.

We describe three patients with cholinergic urticaria in whom we could provoke both the angio-oedema and urticaria by physical means. All patients were investigated by assessing clinical response, blood histamine levels by bio-assay and pulmonary function after physical challenge.

PATIENTS

Case 1

A 34-year-old man was referred with the diagnosis of cholinergic urticaria and idiopathic angio-oedema. Over a 2-year period he developed a widespread, itchy, short lived, small papular rash and eyelid swelling occurring on energetic swimming, on playing squash and after a hot bath. There was no tongue or lip swelling or associated symptoms. He had mild eczema and asthma until the age of 14 years. Family history was not contributory. General examination was normal. Results of tests for dermographism, as well as ice-cube, water and hot beaker (45°C) challenges were negative or normal. Full blood count, liver function tests, urea and electrolytes, proteins and chest X-ray were normal. Complement levels C_3, C_4 and C_1 esterase inhibitor were all normal. α_1 Antichymotrypsin level was 0·62 g/l (normal range 0·4–0·6).

Case 2

A man of 18 presented with a 5-year history of small itchy weals associated with lip and eyelid swelling, occurring initially on severe exertion and more recently after brisk walking, a warm bath, embarrassment and stress. Attacks occurred up to seven times per day, and were associated with a dizzy sensation. His mother had asthma, hay fever and a similar rash in her late teens. General examination was normal. Results of tests for dermographism as well as ice, hot beaker (45°C) and water challenges were negative or normal. Investigations showed evidence of recent subclinical infectious mononucleosis with atypical mononuclear cells, a strongly positive Paul Bunnell reaction, raised transaminase levels (aspartate 75 iu/l, normal range 5–35; alanine 115 iu/l, normal range 5–35), and bilirubin 19 mmol/l (normal range 4–16). Further investigation was delayed for 2 weeks. Subsequently proteins, rheumatoid factor, chest X-ray, urea and electrolytes were normal.

Complement levels showed that C_3 and C_4 were normal. C_1 esterase inhibitor level was 0·42 g/l (normal range 0·15–0·26), perhaps being elevated as an acute phase reactant.

Case 3

A thirteen-year-old female presented with a 2-year history of typical cholinergic urticaria, developing after exercise, a hot bath or emotional stimuli. For 12 months she developed increasing severity of the rash with angio-oedema of the face, and associated systemic symptoms of nausea, headache and slight wheezing. She had severe hay fever for 6 years, and there was a history of mild cold urticaria in her mother and grandmother.

Challenge with an ice cube for 15 min produced wealing on her forearm. Testing for dermographism was negative. Full blood count, liver function tests, urea and electrolytes, serum proteins, complement levels C_3 and C_4 and C_1 esterase inhibitor were all normal.

Cold agglutinin, cryoglobulin and cryofibrinogen levels, and mycoplasma antibody titres were not raised, and VDRL and Australia Antigen were negative.

METHOD

The three patients presented above were admitted to St John's Hospital for Diseases of the Skin for further investigations. None of the patients had received aspirin, other non-steroidal anti-inflammatory agents or antihistamines for at least 1 week prior to testing.

Exercise testing was accomplished by the patient running on the spot and using an exercise cycle. Body core temperature was also raised by immersing the patient in a bath at 43° for 20 min. Peak expiratory flow rate was measured with a Wright's peak flow meter; two readings were taken and averaged. Blood samples were taken via an indwelling catheter, at rest, from the start of clinical challenge, and at regular intervals thereafter. Samples were immediately analysed for pharmacological activity. The bio-assay of venous blood was carried out by a modification of the cascade superfusion method of Vane (1964). For cases 1 and 3, three preparations of the myenteric plexus longitudinal muscle were made from adjacent segments of guinea-pig ileum and suspended under 0·4 g tension in three organ jackets arranged vertically in series. The preparations were superfused with Krebs solution of the following composition (mM): NaCl, 118; KCl, 4·7; $CaCl_2$, 2·54; KH_2PO_4, 1·18; $MgSO_4$, 1·18; $NaHCO_3$, 25; glucose, 11. This solution also contained atropine (10^{-6}M), indomethacin ($2{\cdot}8 \times 10^{-6}$M) and methysergide ($4{\cdot}3 \times 10^{-7}$M). In addition, the second and third preparations were treated with mepyramine ($2{\cdot}5 \times 10^{-7}$M). In a second arrangement preparations of rat fundic strip, rat aortic strip and rat colon were set up. These organs were superfused with Krebs solution identical to that superfusing the ilea, but in addition contained mepyramine ($2{\cdot}5 \times 10^{-7}$M), propranolol ($6{\cdot}76 \times 10^{-6}$M) and phenoxybenzamine ($2{\cdot}9 \times 10^{-7}$M).

The contractions of all preparations were recorded isometrically and displayed on a Devices heat-pen recorder. The biological activity due to blood samples was compared with that due to an equal volume of a variety of standard drug solutions which were dissolved in Krebs solution identical to that bathing the test tissue. The volume of blood or standards added to the superfusate was 300 μl and additions were made during continuous flow. Addition of 300 μl of Krebs solution produced no significant response. Standard solutions used were histamine acid phosphate, substance P, prostaglandins E_2 and $F_{2}\alpha$ and bradykinin (base). Dilutions were made up fresh on the day of the experiment.

In case 2, two preparations of guinea-pig whole ileum were used. Both were superfused with Krebs solution containing atropine, indomethacin and methysergide as above, and one treated with mepyramine as previously.

Responses to blood samples which were blocked by mepyramine on the second and third ilea were considered to be due to histamine. The amount of histamine in these samples was determined by bracketing the response to the unknown sample between greater and lesser responses to standard solutions of histamine.

RESULTS

Case 1

The patient cycled vigorously for 10 min, and this was followed 7 min later by the development of multiple 1–3 mm urticarial papules on the chest and back. The papules were maximal at 20 min and lasted 40 min. A hot bath challenge raised the oral temperature from 37°C to 38·2°C. This produced profound urticaria on the face, trunk and arms and legs and angio-oedema of the eyelids and on the upper arms. An audible wheeze developed at 55 min, the peak expiratory flow rate fell from 640 l/min before challenge to 400 l/min at 82 min after challenge (Fig. 1). After a latent period of 55 min after a hot bath challenge, a marked increase in smooth muscle contracting activity was seen in venous blood samples (Fig. 1). The bio-assay showed that the responses to histamine standards were always antagonized by mepyramine, therefore it was concluded that any remaining response to blood in the presence of mepyramine and the other antagonists contained in the Krebs solution was due to a mediator other than histamine, acetylcholine (ACh), 5-hydroxytryptamine (5-HT) or an agent which releases prostaglandin from the assay tissues. Unlike the responses to histamine standards, the responses to blood of the ilea were not antagonized by mepyramine on the second ileal preparation. In this respect, the responses to blood were mimicked by those to prostaglandins E_2 and $F_{2}\alpha$, bradykinin (BK) or substance P (2 pmol). However, the contractions to the prostaglandins, BK and substance P were always slower to relax to baseline after the peak of contraction than those after the addition of blood. Since specific antagonists to these prostaglandins, BK or substance P receptors are not available, further information on the identity of the smooth muscle contracting activity is not available. This non-histaminic smooth muscle activity rose to a maximum at 55 min after exercise and returned to base level at 75 min (Fig. 1). On the rat preparations, the presence of antagonists in the perfusing fluid prevented any actions due to ACh, histamine, 5-HT, catecholamine or prostaglandin releasing activity. The rat stomach strip was sensitive to low concentrations of substance P and the prostaglandins but did not contract in response to BK or blood samples; conversely, the spontaneous activity of the colon was inhibited by both BK and blood samples, although this action was difficult to quantify. Finally, blood samples caused a

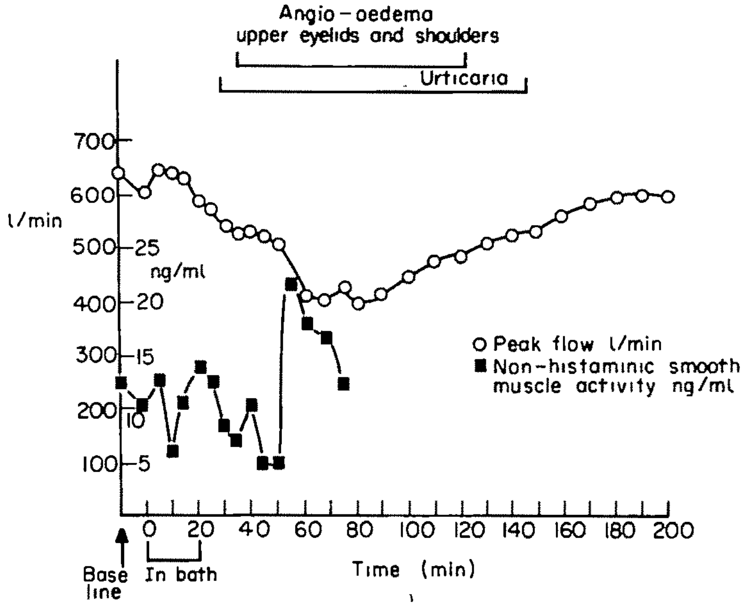

FIGURE 1. Case 1. Hot bath stimulus (43°C for 20 min).

small biphasic response, contraction followed by relaxation, of the rat aortic strip; this action was not mimicked by any of the standards tested.

Case 2

Two experimental challenges were performed on this patient because of technical problems during the first challenge. In the first challenge, running on the spot produced small urticarial weals on the patient's chest, back and forearms 6 min after the start of exercise. At 12 min sheeted urticaria developed on the back and abdomen, both lips swelled and angio-oedema developed on the arms and upper eyelids. The maximum clinical response occurred at 25 min. Histamine levels remained less than 1 ng/ml throughout the experiment apart from a peak of 2·8 ng/ml at 20 min, which was attributed to a technical error with the bio-assay. Exercise challenge was repeated 4 days later (Fig. 2). Running on the spot for 8 min produced widespread small itchy papules on the trunk and limbs, maximal at 20 min. Angio-oedema did not develop. Peak expiratory flow rate remained static. Increased smooth muscle contracting activity was detected which rose from a baseline (pre-exercise) value of 1 ng/ml histamine-equivalent to 20 ng/ml histamine-equivalent at 20 min (Fig. 2). Even at 70 min after exercise challenge, this activity was greatly increased (Fig. 2). That this activity was in fact due to histamine was confirmed using the specific histamine antagonist mepyramine.

Case 3

This patient was challenged by running on the spot for 15 min in a temperature controlled room at 40°C and 70% humidity. Seven minutes after start of exercise, she developed numerous weals on her shoulders, back and trunk. These coalesced from 10 min to form sheeted urticaria and angio-oedema of her abdomen and inner aspect of the thighs. She developed tightness of her chest, but no audible wheeze at 30 min from the beginning of her exercise. Peak expiratory flow

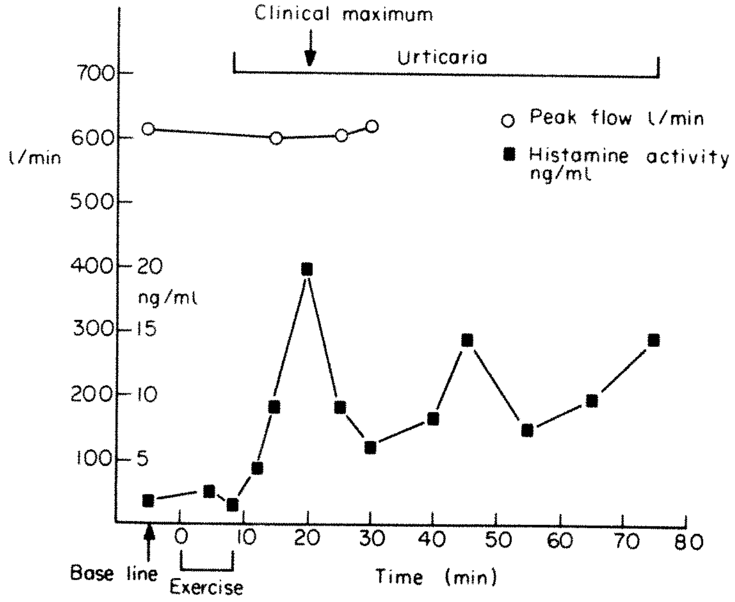

FIGURE 2. Case 2. Exercise challenge (running on the spot).

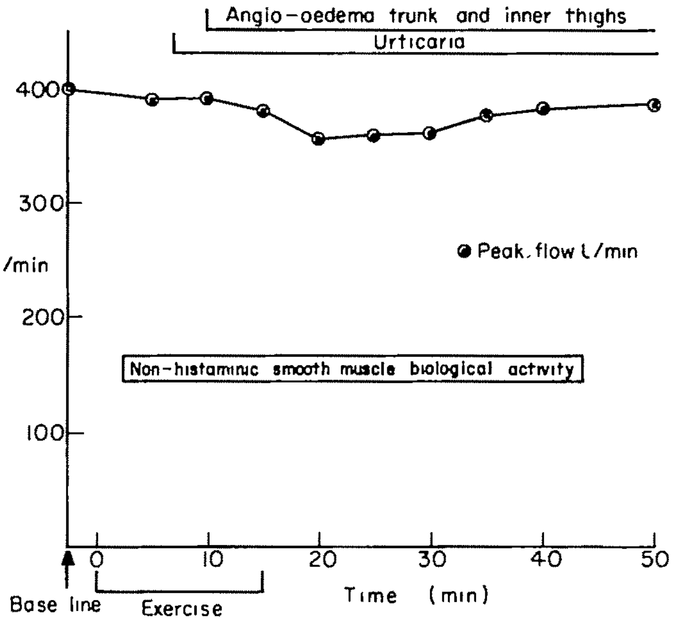

FIGURE 3. Exercise challenge (running on the spot at 40°C, 70% humidity).

rate showed a slight fall from 400 l/min before challenge to 355 l/min at 20 min after challenge, i.e. a change of 11%. Bioassay results resembled that of patient 1, in that there was no increase of histamine levels after challenge. However, from 5–40 min after challenge, there was smooth muscle contracting activity (Fig. 3) of the rat stomach equivalent to 13 ng/ml PGE_2, and relaxation of the rat duodenum greater than bradykinin 40 ng/ml. This activity, however, was not due to bradykinin since doses of bradykinin required to contract the stomach preparation caused a much greater response than the samples on the rat duodenum, and also contracted the ileum. Similarly, doses of prostaglandin E_2 which caused equivalent contractions as the samples on the rat stomach strip and relaxation of the duodenum, caused a measurable contraction of the ileal preparations, unaffected by mepyramine or FPL 55712 and not seen after blood samples. However, the responses to blood samples were only inhibitory on the ilea.

DISCUSSION

The occurrence of angio-oedema precipitated by factors which increase body core temperature in patients with cholinergic urticaria does not appear to have been reported since 1938 (Hopkins *et al.*, 1938). We report three patients with angio-oedema associated with cholinergic urticaria. The appreciation that angio-oedema can be induced in patients with cholinergic urticaria will avoid diagnostic confusion; failure to do so, as in Case 1, could lead to unnecessary investigation and possibly inappropriate treatment.

Angio-oedema may occur in essential acquired cold urticaria (Illig, 1973), acute allergic urticaria and chronic idiopathic urticaria (Champion *et al.*, 1969). It is a major feature of congenital C_1 esterase inhibitor deficiency. Cholinergic urticaria may be associated with cold contact urticaria (Illig & Heinicke, 1967a), dermographism (Morgan, 1953; Herxheimer, 1956) and aquagenic urticaria (Shelley & Rawnsley, 1964). A careful history is important as several stimuli may precipitate different physical urticarias. Cholinergic angio-oedema is diagnosed when other causes of angio-oedema have been excluded in a patient with cholinergic urticaria. Hereditary angio-oedema is ruled out by a careful personal and family history, and a normal level of C_1 esterase inhibitor. Chronic idiopathic urticaria with angio-oedema is excluded by demonstrating the reproducible and close association between the development of characteristic lesions and exercise or a rise in core temperature.

It is interesting to speculate on four possible pharmacological mechanisms of angio-oedema in patients with cholinergic urticaria:

(1) Quantitative differences may exist, with more of the mediators which are normally associated with cholinergic urticaria being released.

(2) There may be qualitative factors; mediators different from those that produce urticaria may produce angio-oedema.

(3) Skin sensitivity to the mediators may be increased.

(4) Non-specific factors aggravating urticaria, such as the ingestion of aspirin, may occur.

The first suggestion is partly supported by the tolerance demonstrated in our three patients. They developed less urticaria and did not exhibit angio-oedema when rechallenged soon after an attack of cholinergic urticaria. Alternatively local immunological factors could explain the development of angio-oedema in some patients with cholinergic urticaria. Further investigations are required to establish whether the angio-oedema in cholinergic urticaria patients is secondary to similar immunological reactions involving the products of eccrine gland stimulation and IgE induced mast cell degranulation, that have been demonstrated in some cases of cholinergic urticaria (Illig & Heinicke 1967c).

Recent investigations (Eftekhari *et al.*, 1980) showed a reduction of α_1 antichymotrypsin in patients with cholinergic urticaria. Hereditary angio-oedema is associated with a deficiency of the inhibitor of the first component of complement. Pharmacologically active products of protein digestion may be involved in the pathogenesis of both cholinergic urticaria and hereditary angio-oedema.

In summary, we have discussed three patients who developed angio-oedema and urticaria after passive overheating and exercise challenge. Peak expiratory flow rate fell in one patient. Blood levels of smooth muscle biological activity rose after clinical challenge in the three patients. In one this was identified as histamine, but in the others we were unable to characterize the active component. It is important to recognize that physical stimuli may produce urticaria and angio-oedema in some patients with cholinergic urticaria.

ACKNOWLEDGMENTS

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Napkin (diaper) dermatitis and gastrointestinal carriage of *Candida albicans*

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SUMMARY

The correlation of recovery of *Candida albicans* from the skin, rectum and faeces of four clinically distinct groups of infants with napkin (diaper) dermatitis has been studied.

We found that: (1) *C. albicans* is largely and constantly present both on the skin and in faeces of subjects with clinically overt Candidiasis of the napkin area; (2) *C. albicans* is lacking from both the skin and faeces of subjects with chafing or atopic dermatitis; (3) *C. albicans* can be found in faeces of subjects with unclassifiable napkin dermatitis in a direct relationship with its clinical severity; (4) failure to recover *C. albicans* from the skin can be due to the inhibitory effect of the inflammatory host reaction and therefore can be misleading.

Napkin (diaper) dermatitis is not a specific diagnosis and is best viewed as a family of disorders due to a variety of aetiological factors. Microbial factors have long been viewed as important in all varieties of napkin dermatitis. The most entrenched view has been the belief that bacteria produce ammonia from urinary urea which then acts as a primary irritant in the napkin area (Cooke, 1921). Recently, we challenged this ancient dogma when we were unable to show any difference in either the amount of ammonia or the prevalence or density of ammonia producing organisms in infants with and without napkin rash (Leyden *et al.*, 1977). In a subsequent study in which we correlated quantitative microbiological cultures with clinical findings we concluded that: (1) the most common form of napkin dermatitis consists of a waxing and waning of erythema, as a result of frictional forces acting on chronically damp skin, and that micro-organisms played no role; (2) in those cases diagnosed as atopic dermatitis, *Staphylococcus aureus* was an important secondary aggravating factor when present in excess of 1×10^6 organisms per cm^2; (3) *Candida albicans* was recovered from 20% of cases diagnosed as seborrhoeic dermatitis and 80% of those diagnosed as 'Candidiasis' but in only 33% of those with the most severe cases of napkin rash with a disseminated 'id' reaction. We recovered *C. albicans* on only five of 145 sites in forty infants free of napkin rash. These results led us to

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conclude that *C. albicans* is an important etiological factor in the more severe forms of napkin dermatitis (Leyden & Kligman, 1978).

Other investigators have also frequently recovered *C. albicans*. For example, Dixon, Warin & English (1969) found an overall prevalence of 41% in 117 patients; Montes *et al.* (1971) reported a 77% recovery rate. In two series dealing with very severe napkin eruptions followed by a generalized 'psoriasiform id', Jefferson (1966) demonstrated *C. albicans* in twenty of thirty-two patients, and Fergusson, Fraser & Grant (1966) reported almost a 50% recovery in twenty-nine similar patients.

In this study, we consider the relationship of *C. albicans* carriage in the gastrointestinal tract to napkin dermatitis.

MATERIALS AND METHODS

Subjects

Two groups of infants with napkin dermatitis were studied. The first group consisted of fifty infants aged 1–15 months. The napkin rash was classified clinically into the following categories: (1) chafing dermatitis (ten cases), in whom the eruption was confined mainly to areas where friction was greatest—the inner surfaces of the thighs, the genitals, the buttocks and the abdomen. This form consisted of a mild erythema with a shiny glazed surface and occasional papules; (2) atopic dermatitis (ten cases)—a variable inflammatory rash associated with typical lesions of atopic dermatitis elsewhere and a family history of atopic disease; (3) Candidiasis—the napkin area showed a confluent intense erythema with a sharp border beyond which were satellite papules or pustules (thirty cases). The area of dermatitis was cultured by the detergent scrub technique of Williamson & Kligman (1965).

In brief, this technique employs two 1-min scrubbings with 0·1% Triton-X-100 in a 3·8 cm^2 area of skin outlined by a sterile glass cylinder. Samples are pooled, serially diluted in half-strength buffered Triton-X-100 and then drop plated onto Trypticase soy agar (TSA), TSA with lecithin and polysorbate 80 Mac Conkey's selective agar for gram negative organisms and Sabouraud's agar. Each infant also had a rectal culture obtained by inserting a sterile cotton tipped applicator moistened with 0·1% Triton-X-100. These samples were processed as described above.

The second group consisted of seventy infants, thirty-five with napkin dermatitis of various forms, and thirty-five free of rash and with no previous history of napkin dermatitis. Classic forms of napkin Candidiasis were not included in this group. In this group there were seventeen females and eighteen males with napkin dermatitis (mean age 42 days) and eighteen males and seventeen females free of rash (mean age 33 days). The napkin dermatitis was categorized by severity, grade 1 consisting of erythema, grade 2 of erythema and papules, grade 3 of erythema and papulopustules, grade 4 of presence of erosions and grade 5 of crater-shaped ulcers and/or nodules.

Quantitative faecal cultures were done in order to correlate the presence and quantity of *C. albicans* in faeces with the severity of the napkin dermatitis. One gram of faeces, directly collected from the napkin, was suspended in 10 ml of saline: 0·1 ml of this suspension and 0·1 ml of each of two tenfold dilutions were plated onto a solid medium and evenly spread with a glass spatula. The medium was Brain Heart Infusion Agar enriched wih 500 gamma/ml of nitrofurantoin to inhibit bacterial growth. *C. albicans* identification was confirmed on the basis of germ tube formation in serum after 2 h incubation at 37°C.

RESULTS

The results of the cultures from both the rash and rectum of fifty infants with either chafing dermatitis, atopic dermatitis and Candidiasis are summarized in Table 1. *Candida albicans* was not recovered from the skin of any infant with either chafing dermatitis or atopic dermatitis. Low number of *C. albicans* (geometric mean of 870 organisms per swab) were found in the rectal culture of five infants diagnosed as having an irritant or chafing type of napkin dermatitis.

In those diagnosed as Candidiasis, *C. albicans* was recovered from satellite pustules in all thirty infants and from the central area of erythema in twenty of thirty cases. The geometric mean count from the area of satellite pustules was 10,240 organisms per cm^2 and 34,120 organisms per cm^2 in the central area of erythema. *C. albicans* was recovered from the rectal culture in twenty-eight of thirty infants and the geometric mean was 18,120 organisms per cm^2.

TABLE 1. Recovery of *C. albicans* from gastrointestinal tract and skin

Clinical diagnosis	Skin	Gastrointestinal tract
Chafing dermatitis	0/20	5/20 (870)†
Atopic dermatitis	0/20	0/20
Candidiasis		
Satellite pustules	30/30 (10,240)*	28/30
Central erythema	20/30 (34,120)*	(18,120)†

* Geometric mean count per cm^2.
† Geometric mean count per anal swab.

In the second group of infants, *C. albicans* was recovered from the faeces of twelve subjects with napkin dermatitis (34%) and in none of the controls. The twelve infants had a geometric mean count of 135·8 organisms/g of faeces. *C. albicans* was not recovered from the faeces of those with mild dermatitis (grades 1 and 2). There was a significant correlation between the severity of the dermatitis and the yeast population in faeces ($r = 0{\cdot}78$, $P < 0{\cdot}001$) (Fig. 1.).

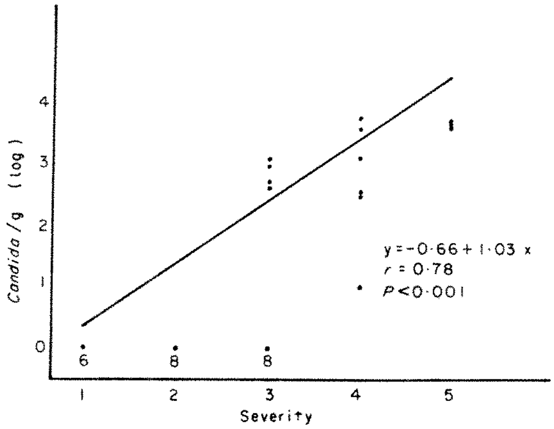

FIGURE 1. Relationship between severity of dermatitis and faecal yeast population.

COMMENT

In this study, we first examined the correlation of recovery of *C. albicans* from the skin and rectum of three clinically distinct groups viz. those with waxing and waning of a mild moderate erythema on contact, frictional surfaces, those with atopic dermatitis and finally infants with typical forms of Candidiasis with confluent, intense erythema with a sharp border beyond which were satellite pustules. These groups were clinically quite distinct and seemed appropriate for investigation of the role of *C. albicans*. *Candida* was not recovered from the skin or faeces of those with atopic dermatitis, nor was it found on the skin of those with chafing form of diaper rash. Five of the latter group did have low numbers of *C. albicans* recovered from rectal cultures. On the other hand, *C. albicans* was recovered from the satellite pustules of all thirty infants with typical Candidiasis and from the rectum in 93%. This result strongly suggests that the gastrointestinal tract serves as a reservoir and that infection results from spread onto the napkin area. *Candida albicans* was recovered from the central area of intense erythema in 66% of cases. This result highlights one of the difficulites in assessing the role of *C. albicans* in any rash. It has been clearly shown in experimental infection models in humans that once intense inflammation and maceration develop, the recovery of *C. albicans* falls off (Rebora, Marples and Kligman, 1973a,b). *Candida albicans* activates the alternative pathway of complement with the subsequent chemotaxis of polymorphonuclear leukocytes and the development of intense inflammation (Ray & Wuepper, 1976). This host reaction appears to be capable of suppressing further growth. The recovery of *C. albicans* from dermatitic skin thus depends on when in the sequence of events cultures are obtained. Cultures during the early phase of the host response (pustules) are invariably positive, cultures from areas of intense inflammation after the activation of complement has occurred may or may not reveal *C. albicans*.

The second group of infants had napkin rashes of a variety of clinical forms but none had the classic form of Candidiasis. They were ranked according to the severity of the dermatitis and our focus was the relationship of dermatitis to gastrointestinal carriage of *C. albicans*. The areas of dermatitis were not cultured in these children because of our previous experience of a variable recovery from dermatitic skin. The results indicated that eruptions consisting of mild erythema with or without scattered papules were not associated with gastrointestinal carriage of *C. albicans*. With increasing severity of inflammation there was a strong correlation with the quantity of *C. albicans* in faeces which suggests that *C. albicans* plays an important role also in the more severe forms of napkin dermatitis which are not identifiable as classic forms of Candidiasis.

Previous investigators have tended to view *C. albicans* as a secondary invader which then aggravates a pre-existing dermatitis. The most complete study in this regard was that of Dixon *et al.* (1969). They studied 117 infants with a variety of clinical forms of napkin rash and cultured the skin and faeces. They recovered *C. albicans* from the skin of 41% and from the faeces in 42%. When they correlated the recovery of *C. albicans* from the skin with clinical impression, they found *C. albicans* in only twenty-seven of forty-three of those cases in whom they felt *C. albicans* was a likely factor and they cultured *C. albicans* in fourteen of fifty-six in whom they felt recovery was unlikely. They considered two possible explanations namely either *C. albicans* was a primary factor and produced various clinical expressions or *C. albicans* was a secondary invader. Because of the discrepancies between their clinical impressions and culture results they leaned towards the latter explanation.

We favour the first possibility because *C. albicans* has long been shown to induce a pustular reaction when applied under occlusion to the skin (Maibach & Kligman, 1962; Rebora *et al.*, 1973a). *Candida albicans* arrives in the napkin area from its natural reservoir, the gastrointestinal tract, and the greater the number of yeasts, the more severe the dermatitis; the same relationship occurs in experimental Candidiasis (Rebora *et al.*, 1973a).

As for the clinical expressions of Candidiasis, it must be kept in mind that *C. albicans* has been recovered from various clinical forms of napkin rash other than the classic form of intense, sharply marginated erythema with satellite pustules, e.g. psoriasiform eruptions (Dixon *et al.*, 1969), seborrheic dermatitis (Leyden & Kligman, 1978), psoriasiform eruptions with generalized 'id' reactions (Jefferson, 1966, Fergusson *et al.*, 1966).

Quantitative isolation of *C. albicans* from faeces of subjects with severe forms of diaper dermatitis should be considered even if the clinical aspects do not suggest Candidiasis.

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Serum α_2 globulin levels in granuloma annulare and necrobiosis lipoidica

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SUMMARY

Alpha$_2$-macroglobulin, caeruloplasmin and haptoglobin were measured in the sera of patients with necrobiosis lipoidica, granuloma annulare and diabetes. α_2 Macroglobulin and caeruloplasmin were significantly raised in diabetes, and caeruloplasmin was raised in necrobiosis lipoidica without diabetes. The ratio of α_2-globulin to serum albumin was significantly high for all three proteins in diabetes, and for haptoglobin and caeruloplasmin in necrobiosis lipoidica. None of these proteins was abnormally raised in non-diabetic patients with granuloma annulare. There is good evidence that the plasma protein changes in diabetes contribute to the development of microangiopathy by their influence on blood viscosity. The altered plasma protein profile in necrobiosis lipoidica may therefore be of relevance to the development of the vascular lesions in this disorder.

The lesions of necrobiosis lipoidica and granuloma annulare are histopathologically very similar, being characterized by focal areas of collagen degeneration surrounded by histiocytes and lymphocytes. Large extravascular deposits of fibrin are a prominent feature and blood vessel walls are often thickened and contain a periodic acid Schiff positive, diastase resistant material. The microangiopathy in these conditions resembles that seen in diabetes (Aagenaes & Moe, 1961; Bauer *et al.*, 1964). Furthermore, necrobiosis lipoidica is associated with diabetes in approximately half of these patients and up to one-third of the non-diabetics show an abnormal reaction to cortisone tolerance or prednisone glycosuria tests (Muller & Winkelmann, 1966; Hill *et al.*, 1966). A similar proportion of non-diabetic granuloma annulare patients would appear to be latent diabetics (Rhodes *et al.*, 1966).

In diabetes, the levels of several plasma glycoproteins are elevated, most notably in the α globulin fractions, and some of these are thought to play a role in the development of microangiopathy. Brownlee (1976) has proposed that angiopathy is due to the accumulation of basement membrane because the abnormally high concentration of α_2 macroglobulin inhibits the activity of proteolytic enzymes which are involved in the normal turnover of basement membrane components. Inhibition of plasmin by the same protein could contribute to the low

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blood fibrinolytic activity in diabetics who develop retinopathy (Almer, Pandolfi & Nilsson, 1975). Haptoglobin and fibrinogen could also impair the function of the microcirculation when present in excess. A strong association has been found between haptoglobin and fibrinogen levels, plasma viscosity and the development of microangiopathy (McMillan, 1976).

Although these blood protein changes are most pronounced when angiopathy is present, it is significant that they may be observed well before the onset of diabetic complications (Cleve *et al.*, 1968; McMillan, 1976; Jonsson & Wales, 1976). To see if similar changes could be implicated in the pathogenesis of necrobiosis lipoidica (NL) and granuloma annulare (GA), serum levels of three α_2 globulins, namely α_2 macroglobulin, haptoglobin and caeruloplasmin, were measured in these conditions.

MATERIALS AND METHODS

Twenty-one patients with granuloma annulare and fourteen with necrobiosis lipoidica, with no apparent abnormality of glucose metabolism (absence of glucose and ketones in urine) or any other disease, were selected for this study (Table 1). They were compared with twenty-three diabetic patients of whom thirteen had necrobiosis lipoidica and four had granuloma annulare. No evidence of nephropathy (proteinuria) was present in any of these patients. Control serum was obtained from thirty-seven healthy members of hospital staff. Since oestrogens may alter levels of α_2 globulins, women taking the contraceptive pill were not included. All proteins were measured by rocket immunoelectrophoresis using antisera from Miles Laboratories and standard serum and plasma from Hoechst Pharmaceuticals. To determine haptoglobin phenotypes, excess haemoglobin was added to serum, and electrophoretic mobility of the haptoglobin-haemoglobin complex was measured on cellulose acetate. Haptoglobin values were

TABLE 1. Clinical details of patients

M/F	No.	Age range (mean)	Duration of disease in years (mean)	Treatment (No.) I = Insulin O = Oral hypoglycaemic agents
Granuloma annulare (widespread)				
M	1	25	1	Hexopal
F	5	49–72(63)	0–20(7)	Hexopal (5)
Granuloma annulare (local)				
M	6	15–53(38)	0–10(2)	Hexopal(2); untreated(4)
F	9	20–63(33)	0·5–5(2·5)	Hexopal(7); untreated (2)
Necrobiosis lipoidica				
F	14	22–71(42)	0–14(5)	Hexopal(9); Bradilan(2) Stromba(1); untreated(2)
Diabetics with NL				
M	4	21–59(46)	5–16(9)	I Hexopal (1) O Bradilan (1); O(2)
F	9	17–45(30)	1–27(10·5)	I Hexopal(5); I(1) I Bradilan(2); O(1)
Diabetics with GA				
M	2	53–74	5–9	O Hexopal (2)
F	2	27–53	0–7	O Hexopal (1); I(1)
Diabetics without complications				
F	6	43–58 (50)	0–20 (12·5)	I (5); O (1)

multiplied by correction factors appropriate to each type which were supplied by Hoechst. Significance of the results was evaluated by Student's t-test.

RESULTS

The mean serum levels of α_2 macroglobulin, caeruloplasmin and haptoglobin were considerably higher in diabetics than in normal controls (Fig. 1; Table 2a). In non-diabetic NL patients, mean haptoglobin and caeruloplasmin levels were greater than in the controls. In GA patients without diabetes no such changes were seen. To eliminate the influence of variations due to sex, the statistical significance of differences in serum proteins was evaluated in females only (Table 2b). The increases in α_2 macroglobulin and caeruloplasmin in diabetes were highly significant as was the elevation of caeruloplasmin in NL. Haptoglobin levels in females with diabetes and NL were approximately 10% higher than normal, but this was not statistically significant.

In diabetics, a depressed albumin level invariably accompanies the increase in the α_2 globulins. Such a decrease was found in this study and was also noted in the NL patients. McMillan (1973) and Jonsson & Wales (1976) have observed that the ratio of glycoprotein

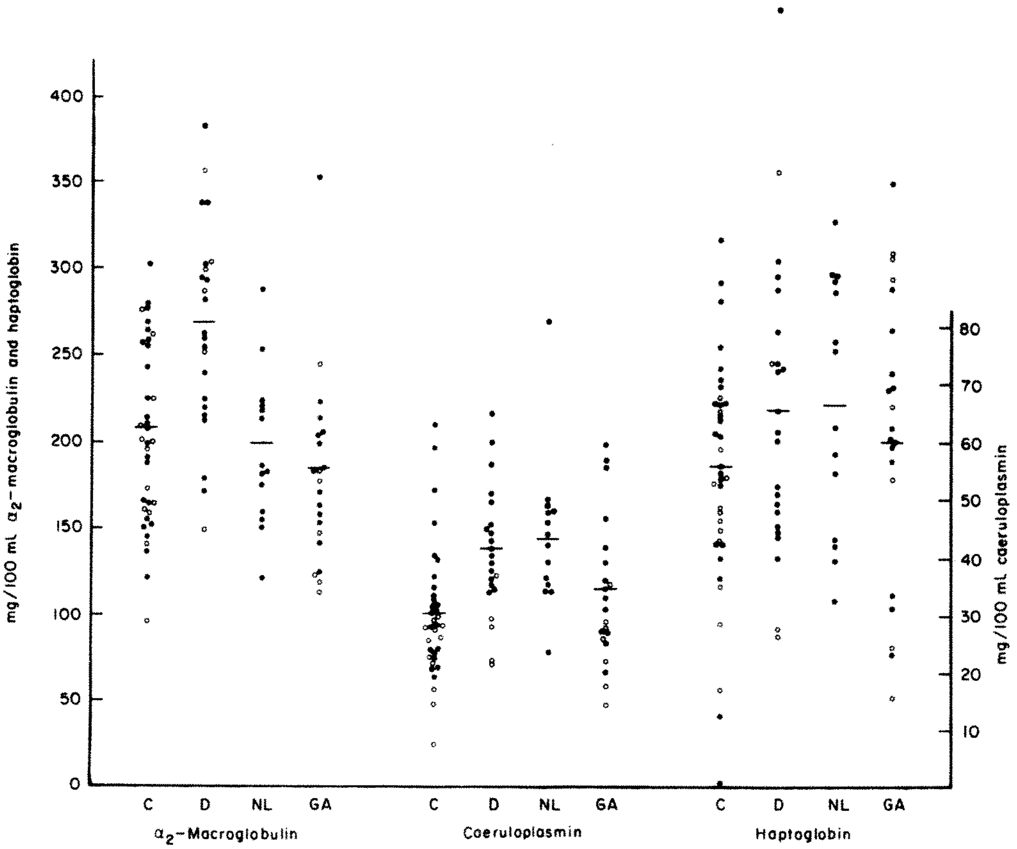

FIGURE 1. Serum levels of α_2 macroglobulin, caeruloplasmin and haptoglobin in patients with necrobiosis lipoidica (NL), granuloma annulare (GA) and diabetes (D), and in controls (C). Open circles represent male subjects. Bars indicate mean values.

TABLE 2. (a) Mean serum protein concentration mg/100 ml ± s.d. (b) Mean serum protein concentration in females mg/100 ml ± s.d. These were analysed separately to eliminate the influence of variations between the sexes

	α_2 Macroglobulin	Caeruloplasmin	Haptoglobin	Albumin
(a)				
Control (N = 37)	203·1 ± 51·2	29·3 ± 11·4	181·8 ± 67·7	4238 ± 503
Diabetic (N = 23)	266·4 ± 59·9	40·7 ± 11·2	217·4 ± 89·7	3915 ± 339
NL (N = 14)	195·1 ± 44·3	43·6 ± 13·1	222·7 ± 73·6	3819 ± 380
GA (N = 21)	180·6 ± 53·6	33·7 ± 12·7	206·9 ± 83·7	4186 ± 275
(b)				
Control (N = 24)	209·8 ± 52·7	32·3 ± 12·1	196·2 ± 72·2	4083 ± 448
Diabetic (N = 17)	263·1 ± 58**	44·8 ± 9·42**	218·6 ± 85·7	3875 ± 388
NL (N = 14)	195·1 ± 44	43·6 ± 13·1*	222·7 ± 73·6	3819 ± 380
GA (N = 14)	191·5 ± 54·8	38·3 ± 12·5	207·1 ± 73·8	4147 ± 256

** Significant difference from control ($P < 0{\cdot}005$).

* Significant difference from control ($P < 0{\cdot}02$).

concentration to serum albumin concentration correlates with the extent of diabetic complications and may even be useful in assessing the likelihood of development of microangiopathy. In view of this, α_2 globulin/albumin ratios were calculated (Table 3). The ratio for all three glycoproteins was significantly raised in the diabetic group, and in NL, caeruloplasmin and haptoglobin ratios were significantly raised.

DISCUSSION

Much attention has been focused on ways in which the increase in certain blood glycoproteins in diabetes could lead to angiopathy. One possibility is the inhibition of enzymes that break

TABLE 3. (a) Mean ratio of serum protein concentration to serum albumin concentration ± s.d. (b) Mean ratio of serum protein concentration to serum albumin concentration in females ± s.d.

	α_2 Macroglobulin	Caeruloplasmin	Haptoglobin
(a)			
Control (N = 32)	49·35 ± 12·21	7·44 ± 3·19	40·94 ± 16·11
Diabetic (N = 23)	68·34 ± 22·66	10·80 ± 3·95	55·48 ± 24·35
NL (N = 14)	53·26 ± 13·38	11·73 ± 3·5	62·62 ± 20·15
GA (N = 21)	42·03 ± 13·31	8·13 ± 3·43	49·72 ± 20·10
(b)			
Control (N = 21)	52·88 ± 11·95	8·51 ± 3·19	45·03 ± 16·9
Diabetic (N = 17)	71·24 ± 22·2***	12·24 ± 3·77***	59·23 ± 24·34*
NL (N = 14)	53·26 ± 13·38	11·73 ± 3·50**	62·62 ± 20·15**
GA (N = 14)	46·03 ± 13·31	9·35 ± 3·43	50·28 ± 18·14

*** Significant difference from controls ($P < 0{\cdot}005$).

** Significant difference from controls ($P < 0{\cdot}02$).

* Significant difference from controls ($P < 0{\cdot}05$).

down the basement membrane (Brownlee, 1976), and of those responsible for the removal of fibrin deposits (Almer *et al.*, 1975). α_2 Macroglobulin, a protease inhibitor, is thought to be of particular importance in this respect. However, the results of this study do not implicate α_2 macroglobulin in the development of the microangiopathy which occurs in necrobiosis lipoidica and granuloma annulare.

Another way in which this change could promote microangiopathy is by its effect on plasma viscosity and hence blood flow. Plasma viscosity is increased in diabetics, especially so in those with angiopathy (McMillan, 1976, 1973). McMillan has found that the proteins whose levels show the strongest positive correlation with plasma viscosity are the acute phase proteins, haptoglobin and fibrinogen, whilst albumin shows a negative correlation. The imbalance in the plasma protein profile in necrobiosis lipoidica observed in the present study could, therefore, be relevant to the development of the vascular lesions in this disorder. High blood viscosity in itself is not likely to cause necrobiosis lipoidica, but could predispose to small blood vessel deterioration in areas of slow blood flow such as the anterior tibial surfaces.

Our results might imply a closer relationship between diabetes and necrobiosis lipoidica than between diabetes and granuloma annulare, i.e. they could reflect a higher incidence of 'latent' diabetes in the necrobiosis lipoidica patients. However, abnormal prednisone glycosuria tests have been reported in both conditions, but as Cudworth (1976) has pointed out, many diseases are associated with abnormalities of carbohydrate metabolism, and it is a problem to define where normality ends and diabetes begins. Other factors could account for the altered protein profiles in necrobiosis lipoidica. For example, both haptoglobin and caeruloplasmin are acute phase reactants whose production is stimulated by various inflammatory conditions, infections, neoplastic disease and psychological trauma, and this is usually accompanied by a fall in serum albumin. Some workers have found that haptoglobin levels rise with age (Bergstrand *et al.*, 1962), although others have not confirmed this (Jonsson & Wales, 1976). Moreover, since there was no corresponding increase of α_2 macroglobulin in the necrobiosis lipoidica patients and because an increase in this protein is linked more strongly with diabetes than with any other non-specific inflammatory reaction (Ganrot, Gydell & Ekelund, 1967; Muller *et al.*, 1970), it seems unlikely that a prediabetic state generated the abnormality in their serum proteins. Even in diabetes the importance of α_2 macroglobulin may be overestimated. The work of Hamlin, Kohn & Luschin (1975) indicates that basement membrane material accumulates in diabetic microangiopathy because of a change in the nature of the collagen which makes it resistant to attack by collagenases rather than because of an inhibition of the enzymes by α_2 macroglobulin. In this connection it is interesting that patients with diabetes and necrobiosis lipoidica had high levels of caeruloplasmin, to which most of the copper in plasma is bound. Both collagen and elastin are stabilized by the formation of intermolecular cross-links in a process catalysed by copper dependent enzymes. Studies are now in progress to see if any alterations in basement membrane components comparable to those in diabetes occur in necrobiosis lipoidica and granuloma annulare.

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Pharmacology and Treatment

Urticaria pigmentosa: clinical picture and response to oral disodium cromoglycate

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SUMMARY

Fifty-six children and adults with urticaria pigmentosa were examined and their symptoms evaluated. Thirteen patients with cutaneous and systemic symptoms consented to participate in a blind trial and were treated for 1 month with placebo and for another month with oral disodium cromoglycate (DSCG). More than 66% of the patients noted relief of pruritus and gastrointestinal symptoms with DSCG. Reduction of wealing was less prominent. Patients have been continued on 400–800 mg of DSCG daily for up to 15 months with continuous improvement of their symptoms and no adverse effects.

Urticaria pigmentosa is characterized by a multifocal cutaneous proliferation of dermal mast cells that becomes noticeable during infancy or in adult life due to changes of pigmentation in the overlying skin. Local or systemic symptoms due to mast cell degranulation may or may not be present and, in rare cases, increased numbers of mast cells may be found in other organs (Sagher & Even-Paz, 1967).

During the past 2 years, we have seen fifty-six patients with this affliction at our hospital. In most cases, the patients sought medical help because of cosmetic problems or because of concern about the nature of their skin eruption. Only about one-third of the patients complained about wealing or pruritus of their skin or of gastrointestinal symptoms, and of this symptomatic group, most patients consented to participate in a blind trial of oral disodium cromoglycate (DSCG) treatment versus placebo. The results of this treatment are presented, together with a brief survey of the clinical picture in this unusually large patient population.

METHODS

The diagnosis of urticaria pigmentosa was made on the basis of a careful history and physical examination and after dermal mast cell proliferation was proven by skin biopsy. Patients with systemic symptoms were, in addition, subjected to a number of tests including gastrointestinal

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endoscopy and bone marrow examinations. Only patients with evaluable symptoms were asked to participate in the trial with oral DSCG. They were informed that two types of tablets were being tested for their effectiveness and were asked to record daily on a flow sheet the severity of pruritus and wealing on a 0–3+ score. Extra space on the flow sheets was provided for the daily recording of other changes such as systemic symptoms due to the primary illness or the therapy. All patients were given white placebo tablets, with instructions to take one tablet three times daily, preferably 30 min before a meal. For the next month, adults were treated with eight capsules daily of oral DSCG (Colimune 100 mg, Fisons Pharmaceutical GmbH), to be taken two at a time 30 min before meals. Children were given only two or four capsules daily, depending on their age. Patients were seen at monthly intervals, and physical and laboratory examinations were performed at that time. Those willing to continue with DSCG beyond the two month blind trial were allowed to do so in an open trial.

RESULTS

Table 1 summarizes the data on all the urticaria pigmentosa patients examined during the past 2 years. There was a slight preponderance of females in both the juvenile and adult type groups. The mean age of onset was less than one with the children, and the duration of disease when the patients were first seen was much lower in this group. Only one juvenile patient continued to have her disease beyond puberty. The lesions in all the cases seen were of the maculo-papular or the papular type, involving trunk and extremities, and in a few cases the face. In about one-third of the patients, the disease was still spreading. Systemic manifestations, consisting of intermittent diarrhea and abdominal pain with flushing, were present in only two children and three adults. About 10% of children and almost 50% of the adults complained of pruritus on contact with physical stimuli such as stroking or changes in temperature. One patient had an increase of mast cells in the bone marrow and another one had hypergammaglobulinemia and an eosinophilia of 10%. Although the development of the urticaria pigmentosa could be dated to a major illness or the taking of contraceptives in a few adult cases, the reason for the onset could not be associated with any specific event in the personal medical history of the patients or during the pregnancy of the mothers.

Only three children and ten adults were admitted to the trial with DSCG, on the basis of evaluable symptoms and their willingness to participate. They were treated for one month with placebo and for the next month with oral DSCG. Table 2 shows the results of therapy. Only one

TABLE 1. Clinical data on fifty-six patients with urticaria pigmentosa

	Children	Adults
Total number	28	28
Male/female ratio	12/16	12/16
Mean age of onset (years)	0·8	26·1
Mean duration when seen (years) ± s.d.	3·9 ± 3·5	8·9 ± 7·0
Patients with spreading disease (%)	36	32
Pruritus (%)	11	46
Systemic symptoms (%)	7	11

TABLE 2. Responsiveness of symptoms to oral DSCG

	No. responding (%)		
	Pruritus	Wealing	GI symptoms
Children	66·7	33·3	100·0
Adults	70·0	70·0	66·7

adult with pruritus felt that she had improved on placebo. An approximately equal percentage of patients in both groups reported relief of their pruritus with DSCG which was confirmed by evaluation of the symptom scores. Wealing was reduced less with the children, and an improvement of the scores was present in only 50% of all patients. Gastrointestinal symptoms were dramatically relieved in three of four patients. At least 2 weeks of therapy were necessary before an effect was noted. Side effects of the drug were not noted by any of the patients. Hematological parameters in the peripheral blood as well as serum enzyme levels remained unchanged during therapy.

All patients who responded to therapy have continued to take oral DSCG, some for up to 15 months. When treatment was stopped, symptoms recurred within 2–3 weeks. The dosage could be reduced with the adults to four tablets daily with continued relief. In the patient with hyperglobulinaemia, the IgG, IgA, and IgM values had returned to normal after 5 months of therapy. Her eosinophilia, however, increased from 10 to 23%. This patient also noted relief of her gastrointestinal symptoms, and she was the only one who had partial regression of her skin lesions. Adverse effects of long term treatment were not observed.

DISCUSSION

In the present study, efforts were made, by scrutinizing the past history and the physical findings of this large group of patients with urticaria pigmentosa, to find a new clue to the pathogenesis of the local and generalized mast cell proliferation. This search was stimulated by our observation in the laboratory that mononuclear phagocytes can be induced to proliferate and differentiate into mast cells under very special culture conditions (Czarnetzki, Hannich & Niedorf, 1980; Krüger, Feldmann & Czarnetzki, 1981). Unfortunately, no common event that might explain the mast cell proliferation was found in the history of the patients. The clinical picture (Table I) shows, however, some interesting features such as the clustering of the onset of the disease during the first year of life and again during the third decade, a slight preponderance of females over males, a low incidence (around 10%) of systemic symptoms in the entire groups and more pruritus in the adult than in the juvenile group.

Based on previous observations that patients with mastocytosis are helped by treatment with oral DSCG (Dolovich, Punthakee & MacMillan, 1974; Völkel & Kruse, 1977; Soter, Austen & Wasserman, 1979), the drug was tested here for the first time in a larger group of patients who had mostly cutaneous symptoms. Pruritus is a symptom difficult to evaluate, and patients tend to vary greatly in their interpretation of pruritic stimuli of the same kind. The almost equal response with the children and adults on treatment with DSCG, compared to placebo, is all the more striking (Table 2). The improvement was readily volunteered by the patients or their parents, and on the score sheets a reduction in the symptoms was clearly evident only after a period of at least 2 weeks of treatment. The one treatment failure among the children was with a girl whose symptomatology was not very well expressed. Similar problems may explain the low responsiveness of the wealing with the children. In general, the most dramatic results were seen with clear-cut, severe symptomatology. This includes the pruritus as well as the flushing, the abdominal pain and the diarrhea. The one adult patient who failed among this latter group had also not obtained relief from treatment with H_1-blockers and several months of PUVA therapy. This patient was rather difficult to deal with during the trial, and her compliance in taking the capsules is open to doubt.

Less than 1% of an oral dose of DSCG is absorbed systemically (Walker, Evans & Richards, 1972). Its high concentration in the gut may explain the favorable results in patients with gastrointestinal symptoms while the poor absorption may account for the lag period before relief of pruritus among the patients with urticaria pigmentosa. The oral form of DSCG has been reported to be effective mainly with gastrointestinal diseases such as ulcerative colitis (Malolepszy, Kuczynska-Sekieta & Chachaj, 1977; Mani *et al.*, 1976), dermatitis herpetiformis (Rhodes, 1978) and gastrointestinal food intolerance (Brostoff *et al.*, 1979; Kocoshis & Gryboski, 1979; Gerrard, 1979). Results with chronic urticaria due to antirheumatics or azo dyes have been disappointing (Thormann, Laurberg & Zachariae, 1980).

The mode of action of DSCG is still uncertain, and differences in organ-, species- and stimulus-specific responsiveness suggest that more than an inhibition of mast cell degranulation is involved (Krüger & Bloom, 1979). A recently observed interaction with peripheral nerve fibers (Altounyan, 1980) might explain the relief of pruritus which occurs without any changes in the histaminuria in the patients (Soter *et al.*, 1979). The improvement of hypergammaglobulinemia and the persistence (Soter *et al.*, 1979) or even the increase of eosinophilia in some patients are further indications that other, as yet poorly understood, pathological processes are influenced by the drug.

At most one-third of the patients with urticaria pigmentosa are in need of therapy because of their cutaneous or systemic symptomatology. PUVA treatment has recently been reported to yield excellent results (Christophers *et al.*, 1978), but has been unrewarding in the three patients treated at our hospital. Similarly, cimetidine, alone or in combination with H_1-blockers, has been found to effectively relieve the cutaneous and gastrointestinal symptoms or urticaria (O'Laughlin & Bredfeldt, 1980; Hirschowitz & Groarke, 1979). Both modes of therapy cannot, however, be recommended for long term therapy because of their potentially serious side effects.

Oral DSCG thus appears to be a useful alternative for patients with urticaria pigmentosa. The side effects of the drug reported so far, such as headaches, insomnia and urticaria (Vaz, Tan & Gerrard, 1978), as well as our own favourable results, would justify even the treatment of patients who are troubled only by pruritus. Negative aspects of oral DSCG therapy include the reluctance of some patients to take so many tablets, and the high cost. Our ignorance about the mode of action of the drug is also disquieting. This drug will have to be tested for even longer periods and with more patients before we can be sure whether it is as safe as it appears. Hopefully, further questions such as its effect on the natural course of mast cell proliferation will be answered by more extensive and prolonged testing.

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Dietary influences on 8-MOP plasma levels in PUVA patients with psoriasis

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SUMMARY

To study the effect of diet on 8-MOP plasma levels, these levels were measured three times in twenty PUVA patients with psoriasis; while fasting, after a low-fat breakfast, and after a fat-rich breakfast.

No significant differences were found between the maximal 8-MOP plasma levels in the fasting condition and the levels after the low-fat breakfast, both peaking 2 h after ingestion. After the fat-rich breakfast, however, the 8-MOP plasma levels were not only significantly lower at 2 h but also peaked later, reaching their maximum at 3–4 h after intake. The drug should therefore be given in a dietetically standardized way. This raises the question whether some cases of therapy resistance can be dietetically influenced.

Psoralen therapy followed by exposure to longwave ultraviolet light (UV-A) is a widely accepted method of treatment for psoriasis. Based on the investigations of Pathak *et al.* (1975), the UV-A irradiation is routinely given 2 h after oral administration of 8-MOP. There seems to be a correlation between the serum concentrations of 8-MOP and the degree of photosensitivity (Thune, 1978; Swanbeck *et al.*, 1979). Psoriatic patients who respond poorly to PUVA treatment seem to have significantly lower 8-MOP plasma levels than do psoriatic patients with an adequate response (Wagner *et al.*, 1979). If there is indeed a correlation between these levels and the therapeutic results, it is of prime importance not only that the UV-A irradiation coincides with the maximum plasma level, but also that this level be as high as possible.

PATIENTS AND METHODS

Twenty patients participated in the study. The 8-MOP (0·5–0·6 mg/kg body weight; Oxsoralen,

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Elder, U.S.A.) was administered orally 2 h prior to UV-A irradiation. Samples of 6 ml of venous blood were taken at half-hour intervals for two and a half hours after ingestion of the 8-MOP. The concentration of 8-MOP was determined by means of electron capture gas-liquid chromatography as described by Van Boven & Daenens (1980), the detection limit being of the order of 1 ng/ml plasma. The 8-MOP plasma levels were determined three times for each patient on three different days: while fasting, after a low-fat breakfast and after a fat-rich breakfast. This was all done in a standardized way.

RESULTS AND DISCUSSION

When the patient fasts and after a low-fat breakfast, the maximum 8-MOP plasma level is reached most often in 2 h, the mean values being 38·6 ± 4·5 ng/ml (s.e.mean) and 41·2 ± 7·4 ng/ml (s.e.mean) respectively. The difference between these two values is statistically insignificant ($P > 0{\cdot}8$), which indicates that a low-fat breakfast will not have an important influence on the 8-MOP absorption compared with a fasting condition.

After a fat-rich breakfast, the 8-MOP plasma levels are different, the mean after 2 h being much lower (16·4 ± 2·9 ng/ml) (s.e. mean) ($P < 0{\cdot}001$). Indeed, the peak is not reached in 2 h but is still rising and is much higher at two and a half hours, when the last sample was taken (Fig. 1).

To find out when the peak actually does occur after a fat-rich breakfast, the plasma levels were measured in an additional eight patients every hour up to 5 h after 8-MOP intake. Again the peak is still rising at 2 h, reaching its maximum after 3 h (57 ± 7·3 ng/ml) (s.e.mean) (Fig. 2). Thus, a high-fat breakfast shifts the peak to the right, though it cannot simply be concluded that the

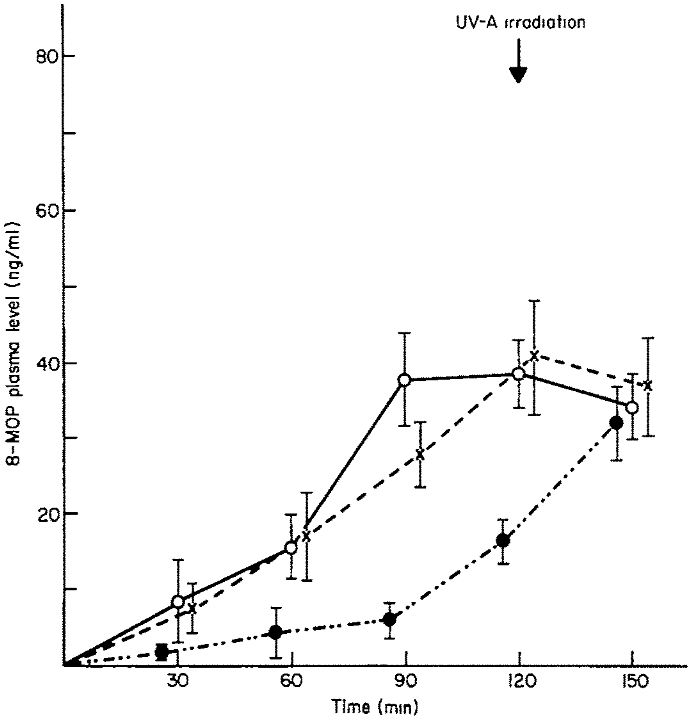

FIGURE 1. Mean values (± s.e.mean) of 8-MOP plasma levels in twenty patients: —— fasting conditions; – – – after a low-fat breakfast; · · – · · – after a high-fat breakfast.

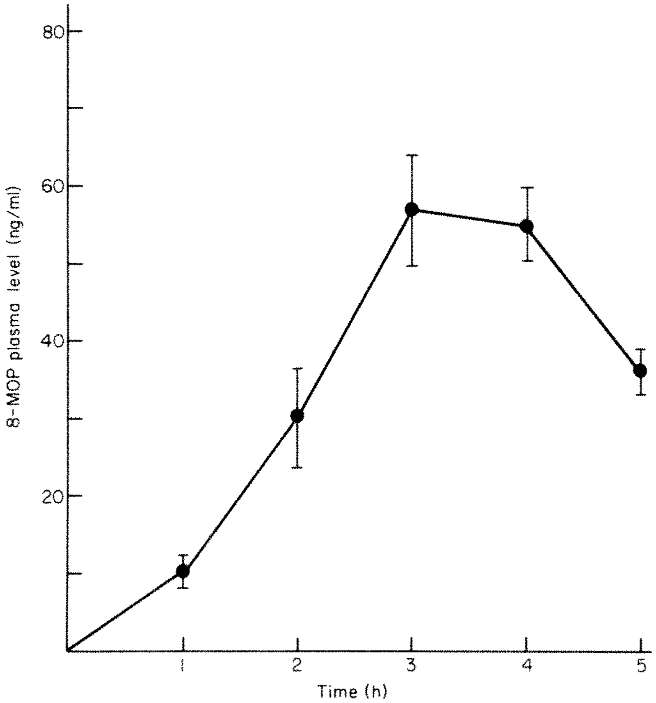

FIGURE 2. Mean values (± s.e.mean) of 8-MOP plasma levels in eight patients after a high-fat breakfast.

mean 8-MOP plasma levels indeed reach a higher peak after a fatty meal, because Fig. 2 and Fig. 1 represent two different groups of patients.

The UV-A irradiation is routinely administered 2 h after intake of the 8-MOP (Parrish *et al.*, 1974; Rogers *et al.*, 1979). If there is a correlation between the 8-MOP plasma level and the therapeutic response to PUVA (Wagner *et al.*, 1979), it is necessary that the irradiation be applied at the optimum moment. When the patients in this study had a fat-rich breakfast, the 8-MOP levels were still low 2 h later when the irradiation was routinely given. It might be advisable, therefore, for psoriatic patients to eat a low-fat breakfast before UV-A irradiation in order to optimize the 8-MOP level. Otherwise, after a high-fat breakfast, the UV-A irradiation should be administered between 3 and 4 h after 8-MOP intake.

This variable could possibly be a major factor in determining the final PUVA results. The drug should therefore be given in a dietetically standardized way. If this is not possible, then the UV-A irradiation should be given not after 2 h but after two and a half hours, when there is practically no difference between the mean 8-MOP plasma levels while fasting, after a low-fat breakfast and after a fat-rich breakfast.

Further research is needed to determine whether some cases of therapy resistance could be based on these findings and if so whether dietary advice could lead to an improvement of the therapeutic results.

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Personal solar UV-A doses received by patients undergoing oral psoralen photochemotherapy for psoriasis

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SUMMARY

Patients undergoing oral psoralen photochemotherapy (PUVA) for psoriasis receive a known amount of UV-A in the treatment cubicle and an unknown amount of UV-A from sunlight. In order to measure the solar UV-A dose, fifty-six patients received a UV-sensitive film badge to be worn from the time they took their psoralen tablet throughout the course of the day, except during treatment. The UV-A doses measured in this way varied from less than 0.2 J cm^{-2} to 7 J cm^{-2}, with a median of around 1 J cm^{-2}. These results show that the solar UV-A received by patients who have been photosensitized by 8-MOP may not be insignificant in relation to the treatment dose.

Oral psoralen photochemotherapy for the treatment of psoriasis is now well established (Wolff *et al.*, 1976; Lakshmipathi *et al.*, 1977; Melski *et al.*, 1977). The treatment, known as PUVA, involves the ingestion of the photoactive drug, 8-methoxypsoralen (8-MOP), followed by subsequent exposure 2–3 h later to ultraviolet radiation in the wavelength range 315–400 nm (UV-A). As yet, PUVA therapy has not been shown to produce a higher than expected incidence of skin cancer although its mutagenic potential has been indicated by the suspected mode of action of the psoralen group of drugs (Editorial, 1978; Epstein, 1979) and observations made on cultured cells (Arlett, 1973) and in animals (Urbach, 1959; Pathak *et al.*, 1959; Griffin, Hakim & Knox, 1959). The only clinical study to date reporting an increased risk of cutaneous carcinoma and the reversal of the normal ratio of basal cell to squamous cell carcinoma (Stern *et al.*, 1979) is open to criticism over (1) the use of inappropriate controls, (2) the inclusion of patients with previous exposure to carcinogenic agents and (3) the failure to record the UV dose levels.

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A psoriatic patient treated with oral psoralen photochemotherapy is exposed to two sources of UV-A resulting in a known dose from artificial sources used in the PUVA cubicle, and an unknown dose, generally to the hands and face, from the UV-A present in sunlight. Both of these sources of exposure will contribute to the photocarcinogenic risk associated with the treatment. A recent survey (Diffey, Challoner & Key, 1980) of the performance of UV-A meters in clinical use has shown wide variations in accuracy, which may suggest that estimates of the 'known' dose of artificial UV-A may be suspect. It is generally recognized that the patient may be exposed to solar UV-A during a period when the drug is photoactive and, for this reason, the patient is advised to wear suitable UV-A protective glasses for the remainder of the day after ingestion of the psoralen to minimise the risk of cataract induction. Also, some workers advise patients to avoid exposure to strong sunshine during the 8-h period after the administration of the 8-MOP tablets (Lakshmipathi *et al.*, 1977), whilst others recommend protection of the skin by suitable clothing, which may include a brimmed hat and gloves (Rogers *et al.*, 1979).

To our knowledge, there is no quantitative information available on the solar UV-A dose (J cm^{-2}) received by patients after they have ingested the drug 8-MOP and while it is still photoactive. Even in the U.K., the level of UV-A irradiance (W cm^{-2}) from the sun can be as high as that in some PUVA irradiation cubicles. Therefore, personal solar UV-A dosimetry has been carried out on patients undergoing PUVA therapy at two centres in the U.K. so that the solar UV-A exposures received can be judged in perspective to the dose of artificial UV-A when assessing the overall photocarcinogenicity of the treatment.

MATERIALS AND METHOD

Solar UV-A exposure was measured using a thin plastic film which incorporates 8-MOP as the chromophore (Diffey & Davis, 1978). When exposed to UV radiation up to a wavelength of 400 nm, the absorption spectrum of the film changes. The observed change in the absorbance measured at a wavelength of 305 nm (ΔA_{305}) can be directly related to the UV dose (J cm^{-2}) received by the film. The film was mounted in a cardboard holder (3 × 3 cm) and worn by the patients attending for PUVA therapy on the lapel site, as this region is judged to receive approximately the same solar exposure as the hands and face.

Fifty-six patients took part in the study (forty in Newcastle and sixteen in Glasgow) which was performed July–September 1980. Patients were instructed to attach the film badge to their lapel at the same time as they took their psoralen tablets and to wear the badge for the remainder of that day. Their clothing is removed during treatment and so the film badge was not exposed to the UV-A lamps used in the treatment cubicle. At the end of that day the film badge was removed, put in an envelope and handed in to the nurse when they next attended for treatment. The film badges issued at both Newcastle and Glasgow were returned to one of the authors (B.L.D.) for spectrophotometric assay.

The dose response of the 8-MOP film was determined by exposing badges to different doses of monochromatic 365 nm radiation and noting the change in absorbance at 305 nm (ΔA_{305}) after irradiation. The radiation source was a 100 W compact mercury arc lamp used in conjunction with a borosilicate condensing lens and a UGI ('black glass') filter, resulting in essentially monochromatic 365 nm radiation plus about 4% of near infrared radiation. Absolute irradiance was determined with a Rank Hilger FT17 thermopile.

The solar UV-A exposure on a given film is related to the equivalent dose of monochromatic 365 nm radiation that would yield the same ΔA_{305} by the expression:

The integration in the numerator is over the UV-A range of wavelengths in sunlight, and in the denominator is over all wavelengths for which the 8-MOP film has a non-zero response. The quotient Q was calculated to be 0·56 (Diffey, 1977).

$$\text{Solar UV-A dose (J cm}^{-2}) = \text{Monochromatic 365 nm dose (J cm}^{-2}) \times Q$$

$$\text{where } Q = \int_{315}^{400} I(\lambda)\, d\lambda \,/\, \int I(\lambda) G(\lambda) d\lambda$$

$I(\lambda)$ is the relative spectral power distribution of natural UVR and $G(\lambda)$ is the spectral response of 8-MOP film normalized to unity at a wavelength of 365 nm (Diffey & Davis, 1978).

RESULTS

A frequency histogram of the recorded solar UV-A doses is shown in Fig. 1. The results have been grouped in intervals of 0·2 J cm^{-2} which is of the order of the limit of resolution. Exposure levels range from less than 0·2 J cm^{-2} to almost 7 J cm^{-2}, 7% of patients receiving doses in excess of 4 J cm^{-2}. The distribution appears approximately log normal with a median exposure of around 1 J cm^{-2}. There was no significant difference between the doses received by patients in Newcastle and those in Glasgow.

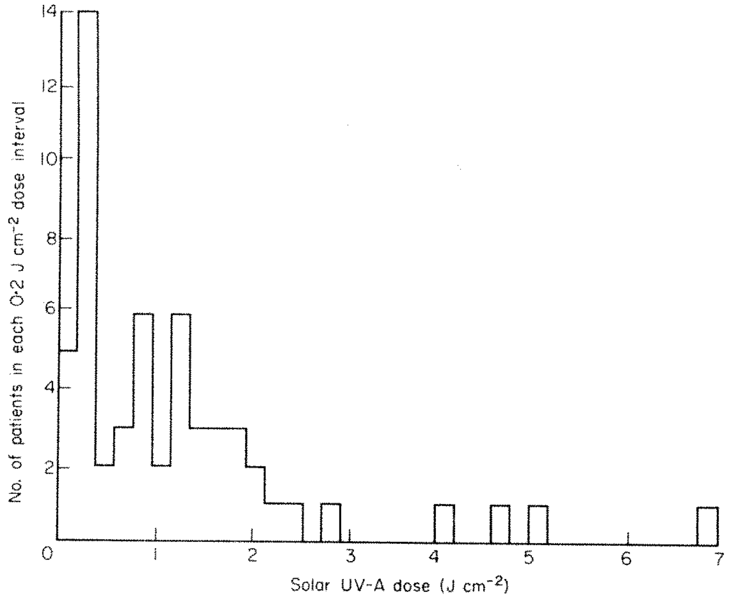

FIGURE 1. Frequency histogram of the personal solar UV-A doses recorded in the study.

DISCUSSION

The photosensitivity of the skin is falling during the day as the psoralens are eliminated from the body. The present measurements do not account for this and consequently will overestimate the biologically-effective UV-A dose. Also, the spectrum of solar UVR is significantly different from the spectrum of the UV-A lamps used in the treatment cubicles (Diffey & Miller, 1980) and strictly speaking the measurements would need to be weighted for the photocarcinogenic action spectrum of psoralens and UV-A, if it were known.

There were at least three contributory factors in the individual solar UV-A doses measured in the present study.

(1) The length of time for which the badge was worn.
(2) The amount of sunshine during the day of measurement.
(3) The behaviour of the patient on that particular day.

The first of these could have been eliminated by having all the patients put on the film badge at the same time of day. However, the purpose of the study was to determine solar UV-A exposure while the patient was photosensitised by 8-MOP without interfering with their normal way of life on a PUVA treatment day. A regression analysis showed that the line of UV-A dose against time at which the badge was put on had a negative slope but the relation was not significant ($P > 0{\cdot}1$). The importance of atmospheric conditions and their variability have been measured (Bener, 1972; Berger, Robertson & Davies, 1975). The environmental UV-A irradiance is partly dependent on the hours of sunshine. Figures were obtained from the Meteorological Office, Glasgow and a regression analysis showed that, although there was a positive gradient on the line relating UV-A dose in Glasgow to hours of sunshine, this was not statistically significant ($P > 0{\cdot}1$). It is relevant to note that the monthly totals of sunshine hours in Glasgow were 123, 109 and 79 in July, August and September 1980, respectively. This is less than the 1941–1970 averages of 159, 143 and 106 and considerably below those found in many other parts of the world. Finally, it is apparent that the main factor contributing to the variability in the UV-A doses was the differences in individual behaviour on the day of measurement.

Clearly the results obtained from this limited study should be interpreted with caution since the solar UV-A doses received by any group of individuals will inevitably show wide variations reflecting the diverse habits of the individuals. Nevertheless we believe the present measurements probably indicate general trends and suggest that patients who are administered oral 8-MOP should be advised to avoid unnecessary sunlight exposure for the rest of that day if solar UV-A doses are to be minimised. These results indicate that solar UV-A received by patients who have been photosensitized by 8-MOP may not be insignificant in relation to the treatment dose, which is of the order of several J cm^{-2}. This should be taken into account when assessing any UV-A dose-dependent aspect of PUVA therapy, such as carcinogenicity or the effectiveness of different treatment regimes, particularly in regions with a higher UV-A irradiance than the U.K.

ACKNOWLEDGMENTS

We are grateful to the nursing staff in the two PUVA treatment centres for distributing the UV-A film badges and to the patients who wore them.

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Case Reports

Speckled hyperpigmentation, palmo-plantar punctate keratoses and childhood blistering: a clinical triad, with variable associations

A REPORT OF TWO FAMILIES

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SUMMARY

Patients from two unrelated families, one from Bristol (two individuals) and the other from Stoke-on-Trent (seven individuals), who exhibited the clinical triad of speckled hyperpigmentation, palmo-plantar punctate keratoses and a blistering tendency particularly on hands and feet in infancy, are recorded. The disorder was inherited as an autosomal trait. The relationship of this clinical triad to other recently reported inherited conditions in which it has been encountered is discussed. A plea for a unified nomenclature is made.

Hyperpigmentation is a feature of many genetically determined skin disorders which may also feature other abnormalities, particularly of ectoderm-derived tissues. The triad of hyperpigmentation, palmo-plantar hyperkeratosis and blistering is seen in dyskeratosis congenita, in the Naegeli-Franceschetti-Jadassohn syndrome and rarely in pachyonychia congenita. However, these conditions have other typical features not seen in our patients, whose particular combination of abnormalities was earlier reported by two of us (Matthews & Peachey, 1977).

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CASE REPORTS

Family 1—Bristol (Fig. 1). Case 1, II 1, K.T., male, born 1944

History. This patient had a normal birth following an uncomplicated pregnancy. Recurrent blistering on the hands and feet developed soon after birth, ceasing at the age of 1 year. Thereafter, punctate keratoses developed on the palms and soles with widespread speckled hyperpigmentation over the rest of the skin. No further change occurred after the age of three years. Physical and mental development was normal.

Family history. His father and mother (now deceased), sister and son have not been affected. However, his daughter (Case 2, III 1) has a similar condition.

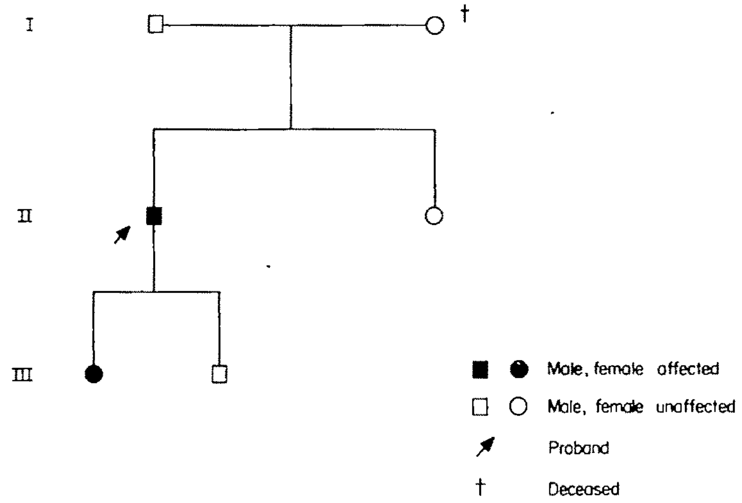

FIGURE 1. Pedigree of Bristol family.

Examination. He is of normal physical development and intelligence. General physical examination revealed no abnormality. He has widespread, ephelid-like hyperpigmented macules over the whole skin including antecubital and popliteal fossae and axillae. The mucosae are normal. The palmar and plantar skin is roughened by multiple punctate keratoses. There is no abnormality of sweating. The teeth, hair and nails are normal.

Investigation. Full blood count—normal.

Family 1. Case 2, III 1, S.T., female, born 1975

History. She was born after an uncomplicated pregnancy and normal delivery. Recurrent blistering of the feet, around the ankles and also occasionally on the hands began in the first few weeks of life, but is occurring much less frequently at the age of 4 years. Blistering does not appear to be related to friction. Speckled hyperpigmentation and punctate keratoses on palms and heels developed at the age of one year. Physical and mental development have been normal.

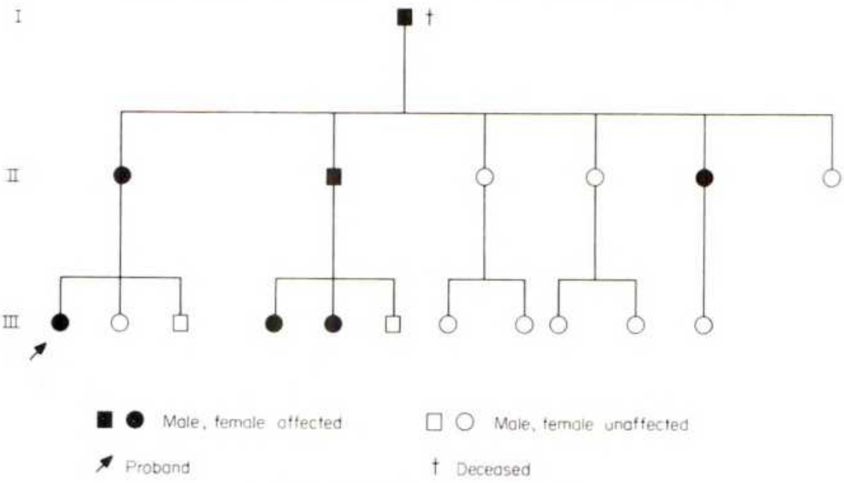

FIGURE 2. Pedigree of Stoke family.

Examination. There is generalized faint, mottled hyperpigmentation, particularly on the limbs. The mucosae are normal. Punctate keratoses are present on the palms and heels. The teeth, hair and nails are normal. There is no abnormality of sweating.

Family 2—Stoke-on-Trent (Fig. 2). Case 3, III 1, J.C., female, born 1967

History. This patient was first seen aged 2 months. Pregnancy and birth had been normal. Blistering had occurred on the feet and hands at the age of 3 weeks (Fig. 3). Hyperpigmentation and keratoses on the palms and soles were first noticed at the age of 10 months.

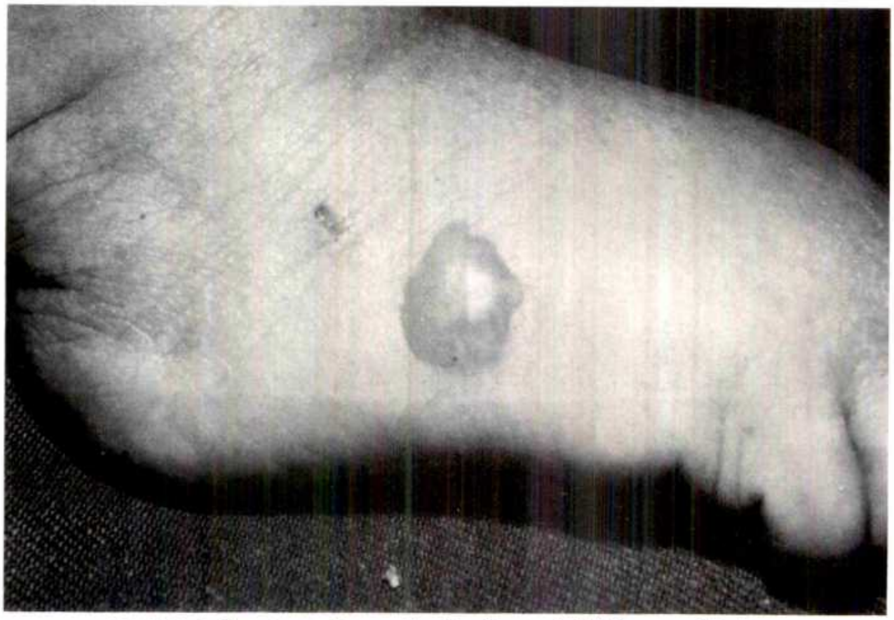

FIGURE 3. Case 3, aged 2 months, showing typical blister on right foot.

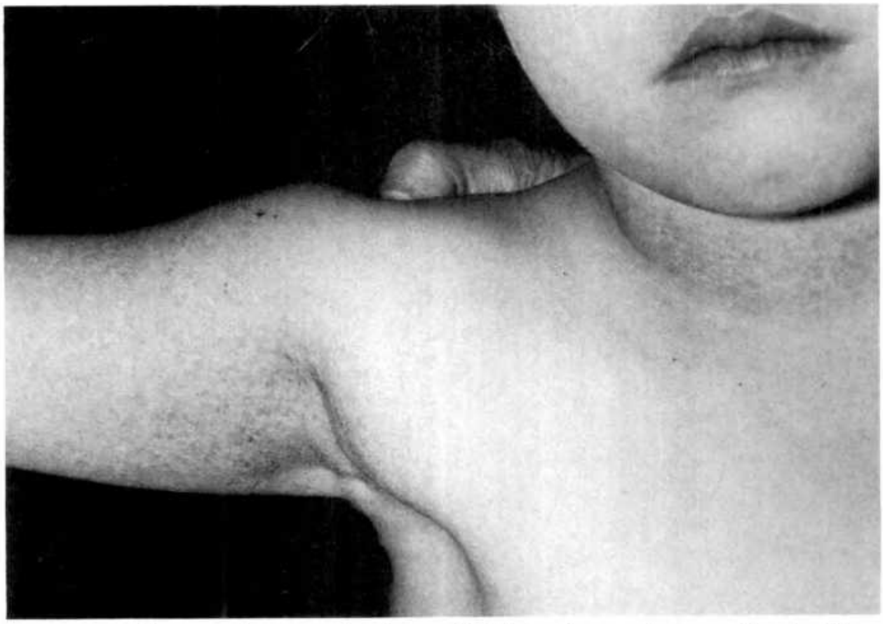

FIGURE 4. Case 3, aged 3 years, showing speckled hyperpigmentation on neck and axillae.

Family history. Her mother, a maternal aunt and uncle, and two female cousins have the same condition. Her maternal grandfather could not recall having had blisters in childhood but showed the other features of the condition when examined.

Examination. On many occasions at follow-up, there were large unilocular blisters on the dorsa of the hands, the toes, the feet and, rarely, on the forearms which healed without scarring. Blistering tended to occur more frequently in the warm weather. There was generalized speckled hyperpigmentation, intensified peripherally (Fig. 4). The mucous membranes were normal. There was no abnormality of the hair, teeth or sweating. When last seen, aged 13 years, blisters were confined to the plantar surfaces of the feet resembling those seen in the Cockayne type of simple epidermolysis bullosa. One finger nail showed minimal terminal onycholysis associated with a 'peaked' lunula. (Nail changes had not been observed before.) Keratoses are present on the palms, the soles and on the backs of the hands, where they resemble plane warts.

Family 2. Case 4, II 1, M.C., female, born 1948

This patient, the mother of Case 3, gave a history of blistering on the hands, shins and feet during the first year of life. Since that time she developed progressive, generalized hyperpigmentation, having a speckled pattern most marked peripherally, and which produced an overall 'unwashed' appearance. There were warty lesions on the palms and soles (Fig. 5). Sweating was normal. The teeth were grossly carious. On one occasion two finger nails showed minimal onycholysis (1–2 mm) associated with 'peaked' lunulae.

Family 2. Case 5, II 2, M.F., male, born 1949

This patient, brother of Case 4, had a similar history of blistering on the hands and feet in

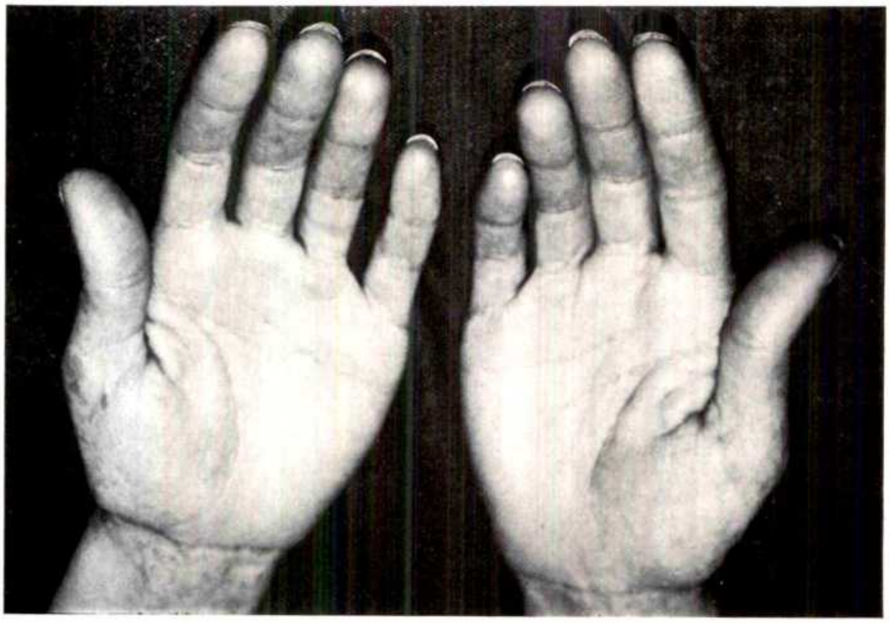

FIGURE 5. Case 4, showing punctate keratoses on the palms, especially evident on the thenar eminences, with speckled pigmentation on palms and flexor aspects of wrists.

infancy, which was followed by the development of pigmentation, and keratoses on the hands and feet. A biopsy performed in 1967 from one of the keratoses on the fingers was unremarkable, with no features to suggest a virus wart. (It has been recently ascertained that this patient's two daughters have developed the complete triad, but they have not been personally examined—Cases III, 4 and 5.)

Family 2. Case 6, II 5, L.F., female, born 1960
This patient was similarly affected.

Family 2. Case 7, I 1, J.K.F., male, born 1922
This patient, father of Cases 4, 5 and 6, could not recall any blistering tendency in childhood, but showed characteristic generalized pigmentation and acrolocated keratoses. He died in 1972, aged 50 years, of chronic bronchitis and respiratory failure.

DISCUSSION

The two families exhibit, in its purest form, a clinical triad of speckled hyperpigmentation, peripherally located punctate keratoses chiefly palmo-plantar, and blistering of the feet and hands; the blistering stage usually confined to infancy. The two families are unrelated. Furthermore, Case 1 had no affected antecedents and, as subsequently the condition was transmitted as a dominant trait, we conclude he is the first recipient of a mutant gene. Both families have been examined by one of us (R.S.) and the similarities confirmed.

The Bristol family was presented to the Bristol meeting of the British Association of Dermatologists (Matthews & Peachey, 1977) where it was concluded that the exact classification of the condition was unclear. However, when it was confirmed that there was another identical family living in Stoke-on-Trent, originally presented to a meeting of the Dowling Club there in 1967, it seemed possible that the syndrome was a distinct entity.

The following conditions have been considered in the differential diagnosis and excluded.

Epidermolysis bullosa

Hyperpigmentation is not a feature of any of the recognised forms of epidermolysis bullosa, nor is the formation of punctate keratoses. A family with dominant simple epidermolysis bullosa is, however, known to us (J.M.B. and R.S.) in which some of the affected members have developed a tylosis-like state of the palms and soles.

Arsenic pigmentation with keratoses

This was considered by the pathologist conducting the post-mortem examination on Case 7 (Fig. 5; I 1), but this suggestion was withdrawn when the family history was made known to him. There was no history of arsenic ingestion in either family and it would be most unlikely that the younger patients had ingested arsenic.

Dyskeratosis congenita

The triad of features under discussion is seen in dyskeratosis congenita. The pigmentation is reticulate rather than speckled, and nail dystrophy, hypoplasia of dermatoglyphics, leukoplakia, blood dyscrasia and alopecia are also typical features. Furthermore, dyskeratosis congenita is inherited as an X-linked recessive (Bryan & Nixon, 1965).

Dermatopathia pigmentosa reticularis (Rycroft, Calnan & Allenby, 1977)

The features of this condition are hyperpigmentation of quite different distribution with deep pigmentation of abdominal skin, lower back and nipples, with the additional features of nail dystrophy and alopecia.

Hereditary acrokeratotic poikiloderma (Weary, Manley & Graham, 1971)

The description of this syndrome is least like that displayed by our cases, but is included for completeness. Hereditary acrokeratotic poikiloderma comprises widespread eczema, vesicopustule formation on the hands and feet in childhood, diffuse poikiloderma, and keratoses of the hands and feet, elbows and knees.

The closest clinical similarities lie between our cases and those in recent studies of Sparrow, Samman & Wells (1976), Fischer & Gedde-Dahl (1979) and Verbov (1980). In these three family studies the clinical triad occurred, but with variable associated features.

Sparrow *et al.* (1976) reviewed *the Naegeli-Franceschetti-Jadassohn syndrome* under the title '*hyperpigmentation and hypohidrosis*' and reported a family, the seven affected members of which showed the basic clinical triad with the added features of hypohidrosis, nail dystrophy, hypoplasia of dermatoglyphics, atrophic changes on the facial skin and dental abnormalities. It is of interest that in our Cases 3 and 4 we were able to detect on one occasion minimal onycholysis associated with angulation of the lunulae.

Fischer and Gedde-Dahl reported another family of eleven almost identical cases under the title '*epidermolysis bullosa simplex and mottled pigmentation: a new dominant syndrome*'. Again, the clinical triad obtained for most of the affected individuals. The blistering tendency in three cases was, however, not limited to the hands and feet and was described as occurring occasionally on the ears, the buttocks, the legs and in the mouth. Milia were present on the feet in one case.

Verbov has most recently reported a family of five affected individuals suffering from '*Hereditary diffuse hyperpigmentation*', in two of whom our clinical triad had occurred. Additional and variable features in this family were spotty hypopigmentation, alopecia, nail dystrophy and smooth finger tips.

As these five families all exhibit, in some of their affected members, this suggested basic clinical traid of speckled (as opposed to reticulate) hyperpigmentation, palmar and plantar keratoses and a tendency to blistering (on the feet usually) in childhood, it seems likely that they are suffering from the same disorder, which is probably transmitted as an autosomal dominant trait, with the onycholysis, hypohidrosis and the other abnormalities being variably expressed. Only in our two families has the basic clinical triad been the sole manifestation.

Other families will inevitably be reported in the dermatological literature emphasising other ramifications, so it is perhaps an opportune time to suggest the desirability of a single title for this disorder.

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Dermatitis artefacta induced in a patient by one of her multiple personalities

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SUMMARY

A young woman suffered for 5 weeks with an inexplicable, severe dermatitis limited to her left arm. During hospitalization it was found that she had multiple personalities, one of whom had been applying the leaves of the poison ivy plant each night to her left arm, using 'his' gloved right hand. The factitial nature of the dermatitis was further documented by the fact that when the 'responsible personality' was cut off from supplies of poison ivy in the hospital, 'he' produced factitious haematomas by trauma to her left hand.

Although this appears to be the first report of dermatitis artefacta associated with the multiple personality disorder, it is suggested that psychiatric study of other dermatitis artefacta patients might reveal the presence of unsuspected occult multiple personalities, responsible for skin lesions the patient cannot explain.

Few medical problems have attracted so much attention as the multiple personality disorder. The concept that more than one 'person' may exist within one body is so foreign to common sense that it is in the twilight zone of the supernatural. But, rare as it is, it is not simply fictional, as in the case of Dr Jekyll and Mr Hyde (Stevenson, 1886). Some 200 patients have been reported in the medical literature (Larmore, Ludwig & Cain, 1977). Three of these, Sally, Eve and Sybil, each has had her startling experience of being inhabited by multiple people dramatically documented in books (Prince, 1906; Thigpen & Cleckley, 1957; Schreiber, 1974), and in eerie movie and television films.

Some scientific observers scoff at the phenomenon of multiple personality, viewing it as *folie a deux*, folly on the part of both patient and observer (Victor, 1975). Nevertheless, psychiatrists generally classify it as a dissociative form of hysteria and thus a type of neurosis. They do not find that the patient is deluding the observer by parading forth a variety of characters in a consummate theatrical performance. On the contrary, all of their tests, including EEGs, support the view of the authenticity of multiple personality disorder (Nemiah, 1980).

The present report describes an experience with one of these patients, in whom not only the

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psychiatric state but the factitial self-induced nature of the dermatitis was initially unrecognized and unsuspected by five physicians.

CASE REPORT

A 28-year-old white woman gave a history of having had a boggy, swollen, eczematous left hand and forearm for the past 4 weeks. The arm was swollen, measuring 28 cm in circumference versus 23 cm for the right arm. It was dusky red and oozing. There were no palpable nodes. Initially the eruption had begun as a vesicular dermatitis, but treatment by four other physicians using topical and systemic steroids had been unavailing, and the process had only worsened. The patient was in good health and her only prior skin disease had been poison ivy dermatitis during her girlhood.

Initially, the possibility of a vascular problem was considered but peripheral vascular studies, including the Doppler, revealed normal circulation. Hospitalization for three weeks included consultations with a vascular surgeon, anaesthesiologist, internist and psychiatrist, as well as general medical studies.

On day 3 and again on day 15 of the hospitalization, a large ecchymotic fluctuant mass appeared on the dorsum of the left hand. X-ray revealed no fractures. Each time 20 ml of blood was aspirated from these. The dermatitis gradually subsided, on a regimen of aluminium subacetate compresses, stellate blocks and compression bandaging.

Psychiatric review revealed that the patient had multiple personalities. Six had been identified but only three were evident during her hospitalization. Each had a name and the ones observed in the hospital were: (1) an unhappy, petulant, pouting little girl of 4 years of age; (2) a belligerent, dominant, macho-type Marine sergeant; (3) the primary patient.

The other three observed elsewhere were an ageing grandmother, a seductive teenager and a college student. On closer questioning, the patient gave a history of a chaotic childhood in which she was the victim of bizarre psychic abuse by a severely disturbed mother.

Further study brought out the fact that the dermatitis began 5 weeks ago when the Marine sergeant wearing a rubber glove on 'his' right hand rubbed on her left arm fresh poison ivy leaves which 'he' gathered on her home grounds. This was done every night and at times the leaves were applied under an elastic bandage. Once in the hospital, the Marine, having no access to the poison ivy plant, turned to episodic traumatizing of her hand, producing large hematomas.

At the time of discharge the skin was healing well and there were no further ecchymoses.

DISCUSSION

Unravelling the nature of this patient's dermatitis was not easy. She presented none of the geometric unnatural destructive lesions which can be recognized at a glance as self-induced. Her problem was limited to the left arm and certainly seemed to reflect a natural disease state. The history suggested a contact dermatitis, but the failure of five doctors to give relief was the first discordant note. This led to hospitalization and the discovery that the patient was indeed a victim of the rare multiple personality disorder. The denouement came with the disclosure that one of her six personalities, the Marine, had while wearing a rubber glove on 'his' right hand regularly applied poison ivy leaves to her entire left arm, covering it with an elastic bandage each night. In turn, with the patient in the hospital and removed from the back yard supply of an antigen to which she was known to be sensitive, the Marine smashed her hand against a metal chair producing large hematomas on two occasions.

It is noteworthy that the personality accounting for the factitial dermatitis and hematomas was a male. Multiple personalities are usually of the same sex as the patient.

One of the most instructive aspects of the study was the observation of the response of a sensitized patient to maximal poison ivy exposure, repeated for weeks. The arm was swollen two to three times normal size, and the epidermis completely denuded leaving a raw red exudative surface. The clinical pattern suggested a vascular blockage rather than a contact dermatitis.

The experience emphasizes the fact that dermatitis artefacta is not always the bizarre and absurd lesion (Naish, 1979). In patients who chance upon techniques of inducing bona fide diseases, detection of the factitial nature may be very difficult if not impossible (Ackerman & Mosher, 1966; Aduan *et al.*, 1979). Indeed, the malingerer with atopic dermatitis could keep a whole pack of physicians at diagnostic bay by discrete exposures to one of his known environmental allergens, such as cat dander.

Finally, these observations raise the question of whether or not other patients with factitial dermatitis may have unrecognized or subclinical multiple personality disorder. Could the self-mutilation be produced by an alternate personality who appears and of whom both the patient and physician have no awareness? In this regard it is well established that the multiple personalities are distinct and unique yet strangers to each other (Ludwig *et al.*, 1972). Moreover, these patients may have real amnesia for what transpires, which is concordant with the factitial patients' claim of no knowledge as to how their lesions arise (Fabisch, 1980). The question lingers, might there be a Dr Jekyll within who can turn and harm their hide?

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Semicircular lipoatrophy

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SUMMARY

Three cases of semicircular lipoatrophy are described.

Semicircular lipoatrophy (lipoatrophia semicircularis) is a relatively new entity, three cases having been recently described by Gschwandtner & Münzberger (1974). Clinically the patients present with horizontal depressions symmetrically distributed on both upper thighs, characteristically on the antero-lateral aspects. We are presenting three cases (the first in the English literature). Cases 1 and 2 are sisters and this is of particular interest because a familial tendency has not previously been reported.

CASES

Case 1

Miss M. L., aged 24, presented in January 1980 with a 15-month history of symmetrical depressions on the anterior aspects of both upper thighs (Figs 1 and 2). They had developed within a few weeks, were asymptomatic and have remained unchanged since. There was no history of trauma but there is a positive family history of diabetes.

Case 2

Miss M. L., aged 25, the sister of Case 1, presented at the same time with a 4-month history of similar symmetrical depressions on the anterior aspects of both upper thighs. They were again asymptomatic. The depressions were, however, not as marked as her sister's and they soon began to show spontaneous resolution.

Extensive metabolic investigations on both patients, including fasting lipids, blood glucose, C-peptides, serum insulin and growth hormone levels in response to an oral glucose challenge, failed to reveal any abnormality. Unfortunately, both patients refused biopsy.

Case 3

Mrs E. W., aged 38, presented in November 1976 with a 6-month history of clinically similar symmetrical lesions just above the knees. There was no history of trauma, they were asymptomatic and again were spontaneously resolving.

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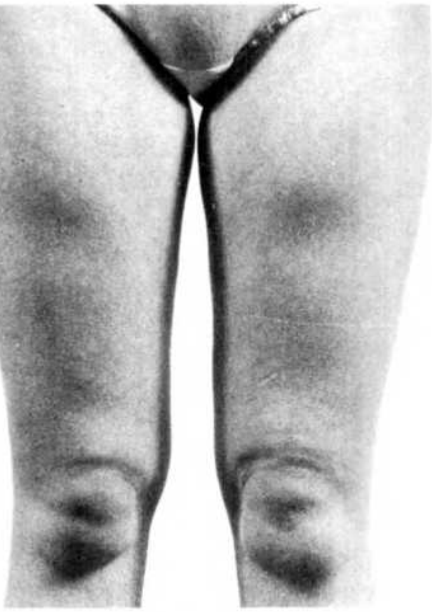

FIGURE 1. Semicircular lipoatrophy in Case 1.

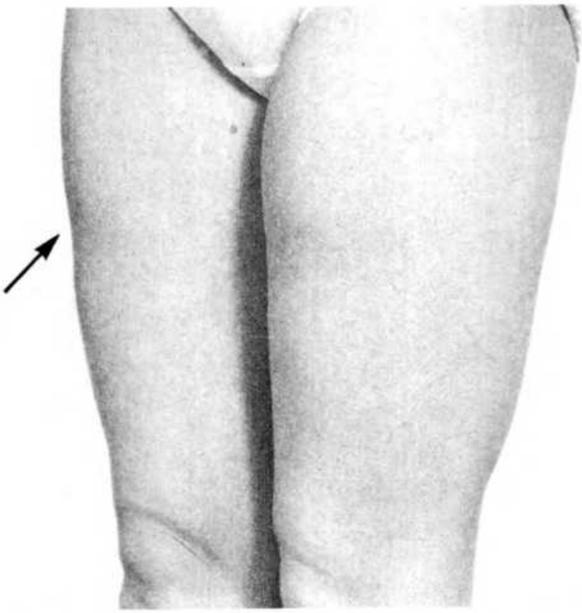

FIGURE 2. Semicircular lipoatrophy in Case 1 (indicated by arrow).

DISCUSSION

Following the description of the first three cases a further nineteen have been published in the German literature (Bloch & Runne, 1978, Gschwandtner & Münzberger, 1975). The history and clinical findings in our cases agree very closely with these. All the patients but one have been female.

In the majority of patients the band-like depressions are 2–4 cm in width and are symmetrically placed on the anterior or antero-lateral aspects of both upper thighs. They characteristically develop rapidly, are asymptomatic and frequently spontaneously resolve. The skin over the affected area is clinically normal. Occasionally the lesions are multiple. Extensive investigations have failed to reveal any obvious aetiology (Gschwandtner & Münzberger, 1975).

Bloch & Runne (1978) speculated that these patients have impaired circulation in the region due to a rare congenital abnormality in the lateral femoral circumflex artery and that repeated local trauma resulted in ischaemic atrophy of the adipose tissue. Histologically their findings were not diagnostic.

Gschwandtner & Münzberger (1975) reported the histological findings in seven patients. There was endothelial swelling and proliferation in capillaries, arterioles and venules with a decrease in the lumen and a perivascular lymphocytic infiltrate. Atrophy of the fatty tissue, destruction of fat cells and replacement by new collagen and an increased number of cells in the subcutaneous tissues were also noted. Unfortunately, our patients refused biopsy and so we cannot add any further information on the histological findings in this condition.

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Erosive candidiasis of the scalp, followed by the reappearance of black hair after 40 years

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SUMMARY

A female aged 72 years developed an erosive pustular dermatosis of the anterior scalp from which *Candida albicans* was repeatedly cultured. The infected area was bald, but on recovery hair regrew and much of this was black although it had been white for the previous 40 years.

CASE REPORT

A female aged 72 years was admitted to hospital in April 1980 with an 8-month history of a worsening scalp skin eruption associated with hair loss. She had been treated with various potent topical steroids and medicated shampoos without improvement. She had been bed- and chair-ridden for many years following a left hemiparesis in 1963.

Examination

She showed a left hemiparesis with poor left limb movement and fixed flexion of the left fingers. The left fingernails were dystrophic and showed yellow-green discolouration, and maceration was present in the third and fourth finger spaces. The scalp showed an erythematous, erosive, scabbed and pustular eruption over the anterior half only, which was associated with alopecia (Fig. 1). The affected scalp skin was atrophic and the pustules were small. The rest of the scalp appeared normal with white hair. Her tongue had white patches over its dorsal surface.

INVESTIGATIONS

A heavy growth of *Candida albicans* grew repeatedly from scalp swabs. A single early swab showed a growth of *Proteus mirabilis* in addition to *Candida*. By late April no growth was obtained. *Candida albicans* was also cultured from the left little fingernail clippings and from a tongue swab.

This paper was presented at a Clinicopathological Meeting of the Dermatology Section of the Royal Society of Medicine on 18 December 1980.

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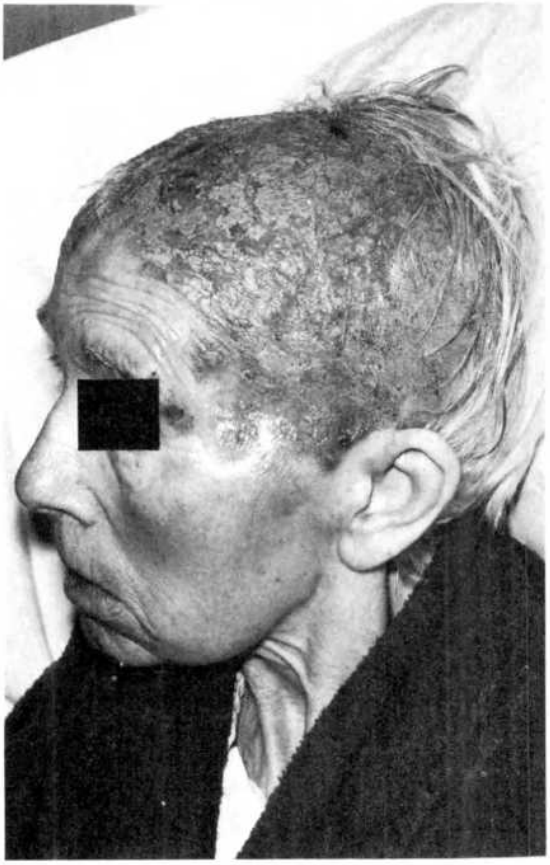
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FIGURE 1. Scalp profile showing erosive candidiasis.

Histopathology of a scalp skin biopsy taken soon after admission showed an eroded epidermis which was slightly spongiotic and contained occasional polymorphs. The upper dermis was oedematous and contained a dense infiltrate consisting of acute and chronic inflammatory cells. The appearances were those of chronic infection. PAS stain showed numerous fungal hyphae in the stratum corneum. A Gram stain was non-contributory (Dr T.W.Stewart).

Treatment and progress

On admission she received oral erythromycin 1 gm daily and topical coal tar and salicylic acid ointment BPC to the scalp, but from mid-April, in view of the swab results, she received oral Nystatin, Nystatin cream to the finger spaces and around the left fingernails, and topical Miconazole cream to the scalp. There was marked scalp improvement within a week and she was discharged home in early May. The tongue and finger spaces were normal by this time. By the end of May the scalp showed slight erythema only with a few scabs.

In mid-July only a few scabs were visible over the scalp and a good regrowth of hair, both white and black, had taken place. She had had no black scalp hair over the anterior scalp for 40 years. Hair over the unaffected posterior half of the scalp remained white. In early September the scalp was excellent and more black and white hairs had grown in the old eroded areas (Fig. 2). The distal portion of the left little fingernail had separated and new nail was growing.

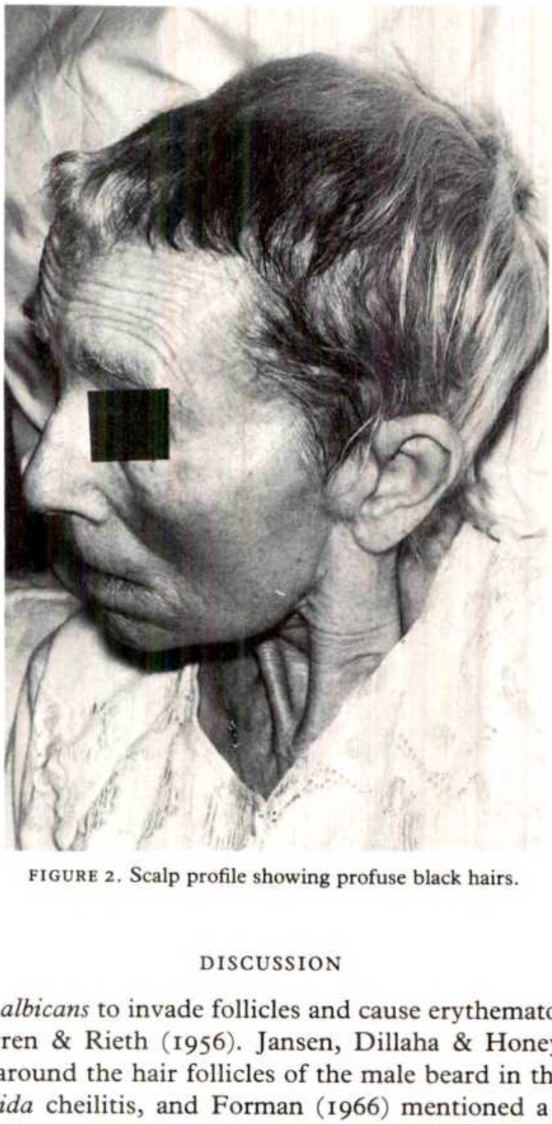
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FIGURE 2. Scalp profile showing profuse black hairs.

DISCUSSION

The ability of *Candida albicans* to invade follicles and cause erythematous pustular folliculitis was reported by Schirren & Rieth (1956). Jansen, Dillaha & Honeycutt (1963) noted an inflammatory reaction around the hair follicles of the male beard in the vicinity of lesions in chronic granular *Candida* cheilitis, and Forman (1966) mentioned a patient with pustular folliculitis around the hair over the back of a hand. The pustules contained abundant hyphae and only *Candida albicans* was cultured from the pus. There has, however, been no previous report of erosive scalp candidiasis, and Maibach & Kligman (1962) were unable to produce scalp candidiasis even experimentally in humans; they thought that inability to completely occlude the scalp was an important factor in their failure to produce experimental infection.

The present patient, who had never had any scalp occlusion, probably transmitted *Candida* from her left fingers to her right hand and thence by touch to the scalp. She showed no response to potent topical steroids and on resolution hair regrew, unlike the patients with non-infective erosive pustular dermatosis of the scalp described by Pye, Peachey & Burton (1979), and by Lovell, Harman & Bradfield (1980), in whom scarring alopecia resulted. My patient, following scalp candidiasis, grew new pigmented hairs in the affected area, having previously had white hair for many years. The black hairs were black throughout their length.

White hair regrowing black may be seen in alopecia areata or following severe systemic illness (Comaish, 1972), but in the patient described it is considered to be post-inflammatory pigmentation. From their experimental work, Maibach & Kligman (1962) considered that cutaneous candidiasis produces an irritant contact dermatitis.

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Nail dystrophy due to sarcoidosis

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SUMMARY

Nail involvement in sarcoidosis is uncommon. It may be a sign of chronic sarcoidosis even in the absence of other cutaneous signs in the affected digits, as illustrated by the following case.

HISTORY

A 65-year-old retired male shopkeeper was referred for an opinion on his dystrophic nails. Sixteen years previously a routine chest X-ray had been reported as showing tuberculous infiltration of both upper zones. His only symptom then was a slight non-productive cough and the chest physician to whom he was referred thought that the tuberculosis was quiescent. No acid-fast bacilli were grown from his sputum and serial X-rays over the next two years showed no change.

He next came under medical supervision 3 years ago when, as a result of a complaint of a persistent cough with mucoid sputum, his chest was again X-rayed. The X-ray showed 'scattered round foci in the upper lobes of both lungs' and he was referred to a second chest physician who also thought that he had quiescent tuberculosis. Recently there was a deterioration in his chest X-ray with extension of the shadowing and it was during an admission for further investigation that an opinion on his dystrophic nails was sought. The patient reported that the nails on two of his fingers had been brittle and thickened for about 7 years. There had been slight soreness of the surrounding skin during that time and he had also noticed increasing redness of his nose and his right great toe.

EXAMINATION

The nails of the left index finger and left thumb were thickened and fragile distally whilst that on the right middle finger was almost lost (Fig. 1). The skin surrounding these nails showed slight scaling with small fissures. The nail on the right great toe had a brown discolouration with

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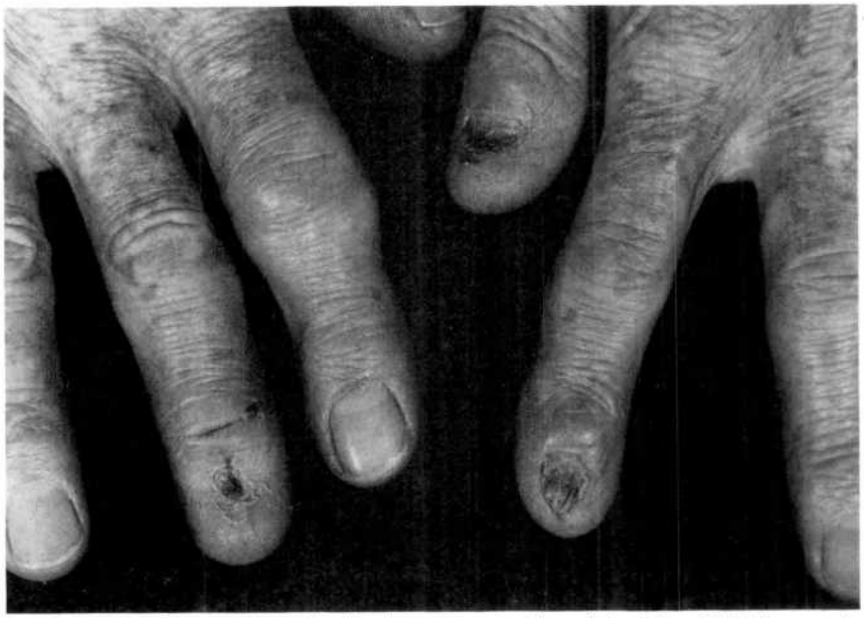

FIGURE 1. Dystrophy of the nails with almost complete loss of the right middle finger nail.

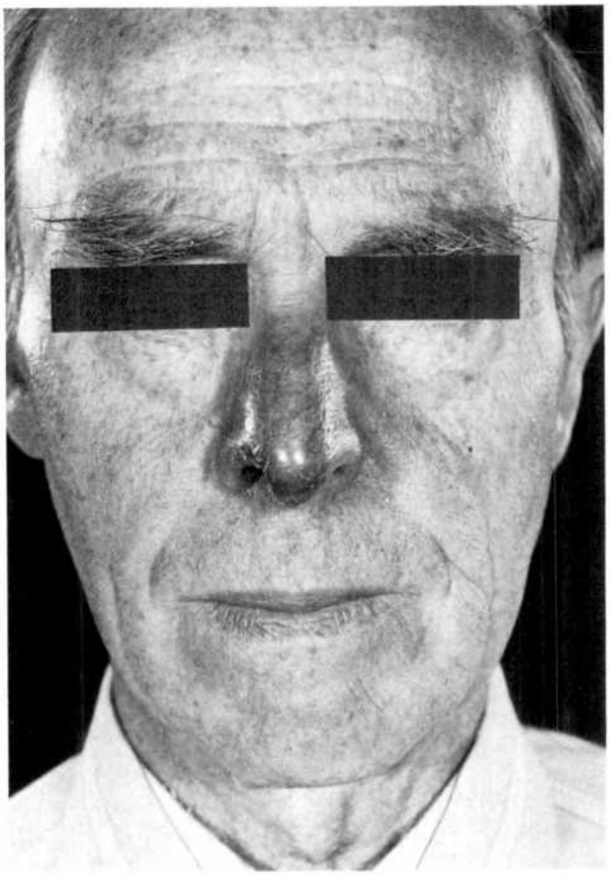

FIGURE 2. The appearance of the nose, showing lupus pernio.

longitudinal ridging and the distal half of the digit was red and scaly. The skin on the nose was telangiectatic, atrophic and erythematous consistent with a diagnosis of lupus pernio (Fig. 2).

General examination revealed swelling of the proximal interphalangeal joint of the right index finger (Fig. 1), bilateral axillary and inguinal lymphadenopathy and scattered rhonchi throughout both lungs.

INVESTIGATIONS

The following investigations were normal or negative: full blood count, serum electrolytes, uric acid, calcium, inorganic phosphate, liver function tests, urinary calcium and phosphate, culture of nail clippings for fungus, and sputum (x8) for *Mycobacterium tuberculosis*. The E.S.R. was 40 mm/h and the Heaf test was negative. A skin biopsy from the nose showed multiple non-caseating granulomata in the dermis and no acid fast bacilli were seen in a Ziehl-Neelsen stained section. A nail biopsy from the right middle finger showed multiple discrete non-caseating epithelioid granulomata with giant cells in the dermis of the nail bed consistent with a clinical diagnosis of sarcoid.

The chest X-ray showed the development of irregular intra-pulmonary shadowing confined to the upper and middle zones of the lungs together with a generalized linearity suggesting fibrosis. The changes were consistent with sarcoidosis. An X-ray of the hands revealed typical features of sarcoidosis with a lace-like reticulated trabecular pattern affecting the phalanges and punched-out cyst-like lesions with thin sclerotic rims in the phalanges. Cortical destructive changes were shown at the tips of the terminal phalanges of the left index finger and thumb and of the right middle finger and these changes corresponded to the affected nails.

DISCUSSION

Lupus pernio is commonly associated with sarcoidosis involving the bones, lungs and eyes (James, 1959), but nail lesions appear to be uncommon. Scadding (1967) and Samman (1978) have described longitudinal ridging, thickening and fragility of the nail plate progressing to loss of the nail in association with lupus pernio of the digit. In such cases the phalanges often show radiological evidence of sarcoidosis. In our patient the finger nails were severely affected by sarcoidosis as confirmed by histology and yet the changes in the surrounding skin were minimal. The patient did, however, have typical lupus pernio of the nose and right great toe.

Considered in isolation the changes of the nails might well have suggested the clinical diagnosis of lichen planus. We suggest that nail biopsy, X-ray of the hands and screening for other evidence of sarcoidosis is advisable in patients with nail dystrophy of unknown cause.

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Cutaneous bullae following acute steroid withdrawal in systemic lupus erythematosus

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SUMMARY

A patient with systemic lupus erythematosus developed severe widespread bullae following sudden steroid withdrawal. Histologically the lesion was suggestive of leukocytoclastic vasculitis with a regenerating subepidermal bulla, but immunofluorescence on non-lesional skin was typical of lupus erythematosus. Treatment led to resolution of the bullous eruption, and despite exacerbations of the patient's lupus nephropathy, the blisters have not recurred. The differential diagnosis of bullous LE is discussed.

Bullous lupus erythematosus (LE) lesions are occasionally seen in systemic LE, but other bullous dermatoses may also occur in conjunction with SLE. Among the bullous conditions reported in association with SLE are bullous and cicatricial pemphigoid, pemphigus, dermatitis herpetiformis, porphyria cutanea tarda, and 'bullous dermatosis of childhood' (Callen, 1980; Jacoby & Abraham, 1979; Penneys & Wiley, 1979; Rothfield & Weissman, 1961; Sunthonpalin & Maguire, 1970). It is therefore essential to formulate a differential in establishing the correct diagnosis when bullae occur in patients with SLE.

Sunthonpalin & Maguire (1970) suggested that the histological appearance of bullous LE was distinctive but others have reported a variety of histological patterns (Penneys & Wiley, 1979; Rothfield & Weissman, 1961). Several authors have failed to find an aetiology for the bullae, whereas Rothfield and Weissman (1961) suggested that sunlight precipitated the vesicular eruption in their two cases.

The reported case developed widespread tense bullae following acute steroid withdrawal, and sunlight was not a contributing factor. The histological appearance was not suggestive of LE, or other bullous dermatosis, but rather a vasculitis. However, the immunological findings were diagnostic of LE.

CASE REPORT

History

This 48-year-old black female was in good health until March 1977, when she noted

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symmetrical arthralgia in her wrists and fingers, pleuritic chest pains and generalized weakness. In June 1977, she developed haematuria and was treated for a presumed urinary tract infection; however the haematuria persisted. In August 1977, she was admitted to Louisville General Hospital because of anorexia, weight loss and intermittent gross haematuria. Evaluation revealed generalized weakness, normal joints and diffuse non-scarring alopecia. Laboratory abnormalities included leukopenia (3200/mm^3), thrombocytopenia, an anti-nuclear antibody titre of 1:640, a low total haemolytic complement, haematuria and abnormal muscle enzymes and electromyography. A diagnosis of systemic lupus erythematosus was made and the patient was treated with aspirin. She was subsequently given prednisone 20 mg per day but in late December she stopped the medication. The patient had continued weakness, intermittent haematuria, and began to notice skin lesions. Over a one-week period she noted progressive skin lesions with blisters on her proximal extremities and trunk. The blisters were not pruritic or painful. Because of progressive weakness, and rapid onset of bullae she was admitted to Norton's Hospital (Louisville, Kentucky) for further evaluation and treatment.

Physical examination

The patient was a cachectic extremely weak female. Diffuse non-scarring alopecia was present. There were no oral or nasal ulcerations. Swelling and tenderness were noted bilaterally in her wrists, metacarpophalangeal and proximal interphalangeal joints. Tenderness was also noted on movement of her knees bilaterally. No effusion or warmth was noted. There was no evidence of joint deformity. The skin revealed multiple intact tense bullae mostly on non-inflamed skin. The lesions were mainly localized to the trunk and neck. Prominent bullae were noted on her breasts surrounding her nipples (Fig. 1). The remainder of the examination was within normal limits.

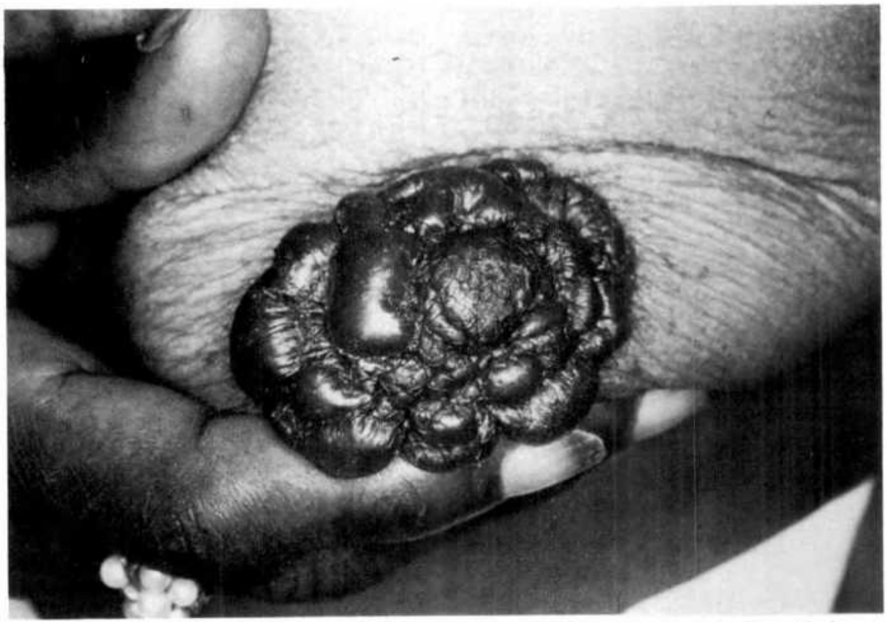

FIGURE 1. Tense bullae on the nipple, with a non-inflammatory base. The surrounding skin is normal. Similar bullae were noted in widespread areas.

Laboratory evaluation

Complete blood count showed haematocrit of 24·0%, haemoglobin 8·6 mg %, white blood count 2600 per mm^3 with a normal differential. Platelet count was 335,000 per mm^3. Westergren sedimentation rate was 62 mm/h (normal, < 20). Blood urea nitrogen, and creatinine were normal. Creatinine clearance was slightly reduced (60 ml/min). Urinalysis revealed many red blood cells, occasional casts, but no proteinuria was noted. Muscle enzymes were normal. Cryoglobulins were not present. Antinuclear factor was 1 : 1280 in a speckled pattern, but test for antibody to extractable nuclear antigen was negative. Anti-DNA (crithedia) was 1 : 40. Total haemolytic complement was normal, however C3 was 35 mg/dl (normal = 123–167), and C4 was 10 mg/dl (normal = 16–44).

A biopsy revealed mild leukocytoclastic vasculitis with fibrin deposits. A non-involved non-exposed area from the arm was sampled for immunofluorescence. The biopsy was strongly positive with granular deposits of IgG and IgM along the dermal-epidermal junction. IgA was not present.

A percutaneous renal biopsy was done and revealed diffuse mesangial thickening and focal glomerulitis. Electron microscopy revealed mesangial thickening, sub-endothelial electron-dense deposits and 'myxo-virus'-like tubo-reticular structures in several endothelial cells. Immunofluorescence showed granular deposits of IgG and complement within all glomeruli. IgM, IgA and fibrin were absent. These findings were diagnostic of lupus nephropathy of the mesangial type.

Course

The patient was initially treated with prednisone 60 mg daily. This resulted in control of her arthralgias, weakness, and weight loss. No further renal deterioration was noted as recently as April 1980, and the patient is now on 20 mg of prednisone every other day. The bullae cleared rapidly and have not recurred, despite occasional symptoms of weakness and arthralgias.

DISCUSSION

This patient met the ARA criteria for the diagnosis of systemic lupus erythematosus (SLE), with non-deforming arthritis, pleurisy, non-scarring alopecia, leukopenia and thrombocytopenia, and haematuria and casts. SLE may have many clinical features but bullae are a rare clinical manifestation (Penneys & Wiley, 1979; Rothfield & Weissman, 1961). Possibly the acute withdrawal of corticosteroids in this patient led to the severe eruption. It is of interest that the patient had no other specific cutaneous manifestations of lupus erythematosus prior to or subsequent to her bullous eruption.

A careful and thorough differential diagnosis must be formulated before assuming that bullae are due to LE. Bullous pemphigoid (BP) could present with lesions similar to those seen in our patient and has been reported in patients with LE (Kumar *et al.*, 1978; Miller, Downham & Chapel, 1978). However, histological and immunological differences would easily distinguish BP. Immunological findings of granular IgG in our case excluded BP blisters (linear staining). In pemphigus the bullae are flaccid, if present at all, and histological and immunological features readily distinguish this process.

Dermatitis herpetiformis (DH) has been reported in patients with LE (Jordan *et al.*, 1969; Moncada, 1974). In addition, bullous LE has been reported to have clinical and histological features of DH. Immunological findings however will readily distinguish DH from the blisters of LE. This patient would not be confused with DH clinically, although one biopsy was initially felt to be consistent with some of the features of DH.

The histological features of this case demonstrate a leukocytoclastic vasculitis. This contrasts with previous reports which showed LE-like histology or DH-like histology. The vasculitis seen in this case was not so severe as to suggest that bullae would result.

Immunological findings in this case demonstrated deposition of IgG and complement in both the skin and kidney. IgM was weakly positive in the skin but absent from the kidney. Previous reports have suggested that it is necessary to have similar immunoglobulins deposited in both sites to ascertain a common pathogenesis for the cutaneous bullae and the systemic LE. This patient demonstrated concurrence of immunofluorescent findings, thus suggesting that her blisters were pathogenetically related to her SLE.

The factors which cause bullae in patients with LE are unknown, but the following have been suggested: (1) increased disease activity, (2) photosensitivity, (3) drug eruptions and (4) steroid therapy withdrawal. This patient developed her eruption after she stopped her corticosteroids abruptly. This led to an increased disease activity, in general, but also possibly allowed the development of her skin rash on otherwise normal skin.

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British Journal of Dermatology (1981) **105**, 607.

Comment

WHICH MOLES MATTER? THE ASSOCIATION BETWEEN MELANOCYTIC NAEVI AND MALIGNANT MELANOMATA

For many years it has been recognized that a proportion of cutaneous malignant melanomata with the superficial spreading or nodular growth pattern arise adjacent to or in association with a pre-existing small melanocytic naevus. This is based both on clinical grounds, as a proportion of patients give a history of an antecedent pigmented lesion present for many years prior to the development of their malignancy, and on the histological observation of residual naevus cells adjacent to the malignant melanoma. The clinical history of a preceding lesion is difficult to evaluate as even reliable patients can be extremely vague about such matters, and the frequency with which adjacent pigmented naevus cells are seen on histological examination varies in individual series but is around 20% (Cochran, 1969).

It is also well established that malignant melanoma may develop in the giant congenital naevi of the 'hairy', 'bathing trunk' or 'garment' variety but once again the frequency of this occurrence is not clear cut as individual series quote a frequency of 2% to 31% of malignant change with a mean of 12% in 54/443 patients (Kaplan, 1974). A prospective register of naevi of this type has recently been started at New York University and it is hoped that this will accurately determine the time risk of malignant change in the unfortunate children with this condition.

A more recent development in the controversy over an association between antecedent benign and malignant tumours of melanocytic origin is the reports of an autosomal dominant condition characterized by multiple large pigmented naevi and a high risk of the development of multiple primary malignant melanoma in these naevi. This condition was reported by two separate groups and named the familial atypical mole-malignant-melanoma (FAMMM) syndrome or the BK mole syndrome (named after the initials of the first two patients. (Frichot *et al.*, 1977; Clark *et al.*, 1978). The clinical hallmarks of this condition are a family history of malignant melanoma and familial occurrence of large moles with an irregular outline and variegated pigmentation. The moles are numerous and found on all body surfaces including covered sites. Histological examination shows evidence of melanocytic activity at the dermo-epidermal junction and the presence of large and sometimes atypical melanocytes within the epidermis. A proportion of such naevi are thought to develop into superficial spreading melanoma and members of such families are reported as having multiple primary tumours. It is of note that despite the striking and characteristic features of this condition and the large number of collaborative clinical groups working on malignant melanoma there have been very few reports of additional affected families since the first two reports. In Scotland, from June 1978 to December 1980, a total of only four such families have been identified in 560 new cases of primary cutaneous malignant melanoma.

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A new development in this suggested interrelationship between melanocytic naevi and melanoma is a report based on retrospective clinical photographic records that 9% (eight of seventy-nine) of all patients with primary malignant melanoma have a pre-existing recognisable condition characterized by multiple (more than twenty-six) irregular or 'ugly' pigmented naevi on all body sites including the few remaining habitually covered sites (e.g. buttocks). These naevi are larger and show greater variation in melanin pigmentation than the normal pigmented naevus. The histological hallmarks of this non-heritable 'naevus syndrome' are reported as being essentially the same as those in the FAMMM or BK mole syndrome with additional recorded features of a lymphoid dermal infiltrate and an increase in collagen bundle thickness in the papillary dermis (Elder *et al.*, 1980).

In the past such naevi when recognized individually have been referred to by pathologists as 'melanocytic naevi showing unusually brisk junctional activity' or more recently 'atypical melanocytic hyperplasia', a term originally suggested for use in situations where a malignant melanoma *in situ* (Clark level 1) which has not penetrated into the dermis is diagnosed. It is now suggested that the term 'dysplastic naevus syndrome—DNS—sporadic type' be used for patients with multiple lesions of this type and that the two genetically determined disorders be renamed 'dysplastic naevus syndrome—DNS—familial type' (Greene *et al.*, 1980).

At the present time the true significance and frequency of DNS whether sporadic or familial is not established. Although normal clinical photographic records are valuable in following sequential changes in slowly growing or changing lesions such as early lentigo maligna, it is impossible to be confident from such records that all pigmented lesions seen are melanocytic naevi, as basal cell papillomata, haemangiomata and even simple lentigines may look remarkably similar on clinical examination (MacKie, 1972). Prospective studies of naevus patterns of all patients presenting with malignant melanoma of skin are therefore needed to establish the true frequency of this condition.

A final report adding to the intriguing but by no means clear cut association between melanocytic naevi and malignant melanoma is a study based on biopsy specimens of melanocytic naevi from patients who had undergone surgical excision of a malignant melanoma. One group of naevi (A) were removed because of clinical concern over development of a second primary melanoma and a second group (B) removed, with the patient's consent, for research purposes. These two groups of naevi were compared with 100 consecutive 'control' naevi (C) removed from patients with no history of malignant melanoma. Evidence of 'activation' of naevi on histological grounds was found in all naevi in group A, 84% in group B and 37% in group C. A most interesting feature of this study was the fact that 60% of 'worrying naevi' in group A were in the area of lymphatic drainage of the primary tumour (Tucker *et al.*, 1980). While this could just be due to morbid preoccupation with the site of surgery, it is unlikely as the patients were examined carefully. It is therefore tempting to suggest that an aetological agent has stimulated a large number of melanocytes in the area in question.

Taking all these reports together it seems clear that the true relationship between benign and malignant melanocytic lesions of the skin remains to be established. While studies on this topic continue, the use of a term such as DNS is a useful abbreviation and probably preferable to other acronyms or 'atypical melanocytic hyperplasia', a term which has come to signify different histological features in various parts of the world. The use of such a label however should not halt the search for the underlying meaning of the association. Do these patients have a specific disorder of DNA repair in cutaneous melanocytes? UV induced DNA repair in lymphocytes is within normal limits in these patients (MacKie & Mackay, 1981) but new developments in tissue culture of normal human melanocytes should enable us to study repair mechanisms in the cells at risk in more detail. Careful investigation of the environmental background of patients with this syndrome is needed with detailed investigation of dietary history, systemic drug ingestion, ultraviolet exposure, and other possible interrelated factors.

For the dermatologist two points are clear: firstly the time honoured statement that melanocytic naevi on the palms, soles and genitalia are more likely to undergo malignant change must be questioned, as the evidence for this is lacking and a recent study of sixty-nine melanomata of palms and soles reported no lesions seen in association with histological evidence of pre-existing naevus (Feibleman, Stoll & Maize, 1980). Secondly, it is clear that follow-up examinations of the melanoma patient after surgery are inadequate if the usual surgical routine of a quick inspection of the graft site and palpation of the draining lymph node basin is all that is practised. All melanoma patients should be examined completely for pigmented naevi at the time of first diagnosis and follow-up should include examination of any detected naevi at this time and removal if sequential photography shows evidence of significant change. Second primary tumours develop in around 10% of melanoma patients and as it is likely that a proportion of those arise in association with dysplastic naevi this routine should in time lower the incidence of multiple primary tumours.

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British Journal of Dermatology (1981) **105**, 610

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Viral transmission of psoriasis

SIR, Mali, De Jongh and Mier in a recent letter (1980), have rejected the viral etiology of psoriasis we proposed (Guilhou, Meynadier & Clot, 1978), arguing that maternal transmission does not predominate over paternal transmission. It is indeed recognized that 'several disorders previously regarded as genetic are in fact caused by the vertical transmission of a virus', but those vertically transmitted viruses are not transmitted in early childhood as stated; such transmission by early exposure to an infectious agent would be a horizontal transmission. In vertical transmission, viral agents are present in the parent's genome and they are transmitted to the progeny through the germ cell (for details see Datta & Swartz, 1979). Thus there is no reason for the viral DNA, behaving as other genes, to be transmitted predominantly in the maternal instead of the paternal genome.

Examples of vertical or horizontal transmission occur in several animal species, including primates. The baboon endogenous virus (BEV) passes to the progeny through the germ cell and therefore it is an endogenous vertically transmitted virus; in contrast, the gibbon leukaemia virus (GLV), transmitted through *in utero* infection or postnatal contact, is an exogenous horizontally transmitted virus (Hirsh, Kelly & Black, 1979).

So, the arguments of Mali and co-workers can be discounted and a possible viral aetiology for psoriasis remains an attractive hypothesis. Beside the fact that vertical transmission of the virus would explain genetic predisposition, enhancement of viral expression could account for cell proliferation and immunological disturbances leading to relapses of the disease.

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Book Reviews

Zinc and Copper in Medicine. Edited by Z.A.KARCIOĞLU and R.M.SARPER (1980) Springfield, Illinois: Charles C. Thomas. Pp. 678. Price $59.75.

At a time when journals of all specialities are carrying an increasing number of papers on the role of trace elements in general, and zinc and copper in particular, a book which aims to put into perspective our current understanding of the latter is particularly welcome. It differs from other recent publications on the subject in that it is not a report of a symposium nor a single author product, but a well edited review of the world literature by twenty-five experts in the field.

Consideration of one sub-speciality per chapter is a helpful format which enables the reader to focus rapidly on his own subject. The editors acknowledge that a certain degree of overlap results, but this is to advantage, for example in the different accounts of acrodermatitis enteropathica by both gastroenterologists and dermatologists. One of the most welcome features, relevant to all workers and fundamental to any trace element research, is a consideration of analytical techniques in the introductory chapters. Zinc as a therapeutic agent has been most widely used in diseases of the skin, and this chapter critically reviews the position of both trace elements in modern dermatology. The different concepts of replacement therapy in supposed deficiency states and pharmacological activity of zinc salts given in excess of normal nutritional requirements is mentioned, but not emphasised sufficiently, in this section.

The book contains one or two minor typographical errors but is otherwise well produced. Although clinicians would find individual chapters of interest the book will be of most use to researchers for whom the extensive bibliography alone makes this publication an essential requirement.

MICHAEL J.HALL

The Management and Prevention of Pressure Sores. ANTHONY BARTON and MARY BARTON (1981) London: Faber & Faber. Pp. 96. Price £2.75.

This important and valuable little booklet should obviously be read by anyone dealing with pressure sores, particularly nurses and doctors working in intensive care units or anybody in charge of geriatric assessment units or units where patients may be unconscious or over-sedated. I found the chapters on thermography and radiometry stimulating and valuable. The chapter on prevention also was helpful. The authors seem to be a little hard on dermatologists and imply that dermatological treatment makes pressure sores worse. I do not know what the evidence is for the statement on page 56 that rheumatic units may contain more than 50% of patients with pressure sores due to the use of anti-inflammatory material for their joint pains, and certainly the statement that any material such as hydrocortisone used parenterally or as an ointment or eye drops and 'many of the anti-inflammatory ointments used by dermatologists prevent pressure sores healing' requires a little further explanation, particularly when it is the weaker steroids that are mentioned and these are not actually applied to the pressure sores.

I presume that this little booklet is intended for nurses rather than doctors but it would certainly help if references were given when statements are made. In spite of this criticism, I am sure this is a useful book on a difficult subject.

SUZANNE ALEXANDER

Das Basaliom. Edited by F.EICHMANN and U.W.SCHNYDER (1981) Berlin, Heidelberg and New York: Springer-Verlag. Pp. 152, 117 illus. (16 in colour). Price DM 84.00.

Our German-speaking continental colleagues sustain a society devoted to dermatological surgery. This book, subtitled 'The Commonest Tumour of the Skin', is based on contributions to the Society's third annual meeting held in Zurich in 1980. It was an interdisciplinary conference and the contributors were Austrian, German and Swiss dermatologists and surgeons representing various specialized interests.

In fact, only half the book is devoted to treatment, and the first seven articles include absorbing accounts of the clinical and histological versatility of basal-cell carcinoma, growth and metastasis, genetic factors, Pinkus' tumour and vulval involvement.

The therapeutic chapters expound the techniques of plastic surgery and radiotherapy and consider the problems of special areas, e.g. the nose, ear and eyelid. Histographic treatment, i.e. detailed histologically controlled extirpation, receives support (including that of an ophthalmologist) and a plea is made for the wider use of cryotherapy. The limited role of chemotherapy is also discussed.

Each chapter carries an English summary: some are models of clarity, others obscure, so that an intending user should have at least a reading knowledge of German. The bibliographies are international and contain useful references perhaps unfamiliar to the more insular reader. The illustrations are apt and the index serviceable.

It can be recommended as an admirably brief but comprehensive review of basal-cell carcinoma, its biology and treatment.

P.J.HARE

Dermatologie. R.DEGOS (1981) (in French) Paris: Flammarion Medecine-Sciences. Two volumes, Pp. 2936, 738 B&W illus. Price £132.00.

This much respected major French text book of dermatology first appeared in 1953. In loose leaf format it has been updated on twenty-four occasions. The publishers have now produced a handsome two-volume bound version of the book as it was after its 1980 updating. It is still largely the work of Professor Degos with help from Professor J.Civatte and Dr S.Blaich. It runs to nearly 3000 pages with 738 mainly excellent black and white clinical and histological photographs. Tables are surprising by their absence.

The classification of diseases, for which anglophiles can find good precedent in the work of Willan, is based on morphology rather than on mechanisms and aetiology. The chapter headings are erythema, papules, vesicles, etc. This nowadays slightly less common approach has both advantages and disadvantages but clearly has its uses when confronted with a clinical problem and indeed provides an interestingly different slant on dermatology.

Some of the pages of text are still apparently original or are certainly the same as in my edition of 1961, which I have much used and treasured. Some whole sections have not been revised for a while; the lichen planus references reach to 1962, lupus erythematosus to 1967, but many sections have been brought right up to 1979. The references are listed in date order and cover especially the French literature but also the English, German, Italian, Spanish and other languages.

The main strength of this book for an English reader must be its vivid clinical descriptions, fine photographs and an ingress into the French literature and the French style of dermatology. The brief historical summaries and the introduction to many eponyms will have their appeal to some.

The suggestions for therapy might be considered slightly old-fashioned and empirical. There is little attempt to explain the mode of action of all the various local and systemic remedies—the art of dermatological prescription ostensibly to the fore.

This book is a remarkable monument from a remarkable dermatologist, and it is reassuring that we can look forward to further 'mises à jour' in due course.

R.H.CHAMPION

British Journal of Dermatology (1981) **105**, 613-614

News and Notices

Twelfth Annual Meeting of E.S.D.R., April 1982

The 12th Annual Meeting of the European Society for Dermatological Research (E.S.D.R.) will be held in the Rai-Congrescentrum, Amsterdam, Holland, 4–7 April 1982.

The central theme of this meeting will be: The Pharmacology of Inflammation'. Invited experts who will deliver lectures related to the theme include Dr B.Czarnetzki (Münster, F.R.G.), Professor D.Dumonde (London) and Professor Lembeck (Graz, Austria), and there will be two workshops on that topic chaired by Professor Greaves (London), Professor Søndergaard (Copenhagen, Denmark) and Dr Wasserman (San Diego, U.S.A.). The major part of the meeting will, however, continue with free communications as in previous years.

Attendance will be open to all members, contributors and guests. Abstracts should be submitted on the official form. No discrimination will be made by the Programme Committee between abstracts related to the central theme and non-related abstracts.

The deadline for submission of abstracts is 1 December 1981. Abstract forms can be obtained from: Herbert Hönigsmann, Secretary-E.S.D.R., Department of Dermatology (I), University of Vienna, Alserstrasse 4, A-1090 Vienna, Austria.

62nd Annual Meeting of BAD, 1982

The next annual meeting of the BAD will be held at Ninewells Hospital, Dundee under the Presidency of Dr W.Frain-Bell, on 1–3 July 1982. Those wishing to present a paper at this meeting should submit an Abstract (6 copies) of not more than 200 words to Dr G.Levene, Hon. Sec., BAD, 7 John Street, London WC1N 2ES, before Friday 19 February 1982.

Society for Cutaneous Ultrastructure Research

The 9th Annual Meeting of SCUR will be held at the University Medical Centre, Leiden, The Netherlands, on April 1–3 1982.

Dermatologists, biologists and other interested scientific workers are invited to participate. For details and registration forms, please write to: Dr B.J.Vermeer, Department of Dermatology, University Medical Centre, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

British Society for Dermatopathology, Winter Meeting, January 1982

The annual meeting of the Society will be held in Bristol on Friday 29 January 1982. For the first time the meeting will follow, and not precede, the meeting of the Investigative Group of the British Association of Dermatologists.

The symposium in the morning will be on cutaneous vasculitis, with Professor Klaus Wolff as the guest speaker. In the afternoon there will be the usual clinicopathological demonstration of cases.

Members will be circulated with further details in due course. Enquiries regarding the meeting, Society membership, etc., should be addressed to Dr D.H.McGibbon, Department of Dermatology, St Thomas' Hospital, London SE1 7EH.

Hair Replacement Symposium

An International Advanced Hair Replacement Symposium will be held in Birmingham, Alabama, U.S.A., on 3–4 February 1982. There will be a faculty composed of dermatologists, otolaryngologists and plastic surgeons. Registration fee: $420.00. Details from D.B.Stough, III, M.D., Program Director, The Stough Dermatology and Cutaneous Clinic, P.A., Doctors Park, Hot Springs, Arkansas 71901, U.S.A.

International Workshop of Investigative Dermatology (IWID) in Kyoto

The Japanese Society for Investigative Dermatology will hold an International Workshop in Kyoto, Japan, 31 May–1 June, 1982, as a 'Post-Congressional Satellite Meeting' to the XVI International Congress of Dermatology. The topics will include photobiology, melanogenesis, immunology, hormones, inflammation, keratinization, virology and bacteriology, electron microscopy and membrane receptors. Applications to attend the Workshop must be received before 31 January 1982. The language for the Workshop will be English.

A travel fellowship of U.S. $200 per person for this 'Satellite Meeting' is being made available for qualifying active foreign participants. Please apply, with an 800 word abstract of your presentation, and curriculum vitae including bibliography for the last 3 years, to arrive not later than 15 December, 1981.

All correspondence should be addressed to the Secretary General for the I.W.I.D.: Professor Yutaka Mishima, Department of Dermatology, Kobe University School of Medicine, 5-Kusunoki-cho 7-chome, Chuo-ku, Kobe, Japan 650.

APPOINTMENT

Dr David Lloyd Roberts has been appointed Consultant dermatologist to the West Glamorgan Health Authority based at Singleton Hospital, Swansea.

He graduated M.B.B.S.(Lond.) from the London Hospital in 1972 and became M.R.C.P.(U.K.) in 1975. He gained experience in general medicine in London and Cardiff, and has also worked abroad in Bermuda and Canada. He was Senior House Officer and Registrar in Dermatology in Cardiff and later Senior Registrar in Stoke-on-Trent.

He has published a number of papers on clinical and investigative dermatology. His main interests now include contact dermatitis, industrial dermatology and the epidemiology of skin disorders.

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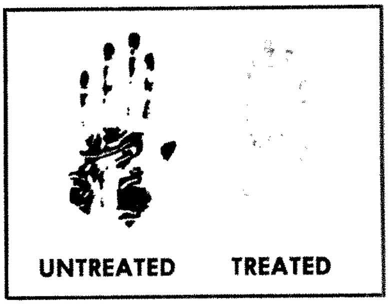

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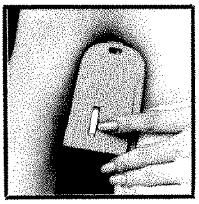

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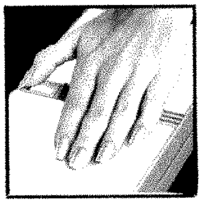

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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British Journal of Dermatology

The British Journal of Dermatology is owned by and is the official organ of the British Association of Dermatologists. It is also the official organ of the Nederlandse Vereniging van Dermatologie en Venereologie (Netherlands Association for Dermatology and Venereology).

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Diseases of the Hair and Scalp

Arthur Rook MA, MD, FRCP
Consultant Dermatologist
Addenbrookes Hospital, Cambridge

and **Rodney Dawber** MA, MB, ChB, FRCP
Consultant Dermatologist
John Radcliffe Hospital, Oxford

This important title, the first work on the subject for a number of years, will be an essential reference for all clinicians involved in the diagnosis and management of diseases concerned with the hair and scalp. It presents a comprehensive account of the hair and its disorders, emphasising their pathogenesis and diagnosis, based on a review of the international literature and the authors' extensive experience.

The first two chapters give a clear account of the embryology, structure and comparative physiology of the hair and the remaining chapters are predominantly concerned with clinical aspects of hair abnormalities.

Contents

The comparative physiology, embryology and physiology of the human hair; Hair follicle structure and keratinisation; The hair in infancy and childhood; Hair patterns: baldness and hirsutism; Diffuse alopecia: endocrine metabolic and chemical influences on the follicular cycle; Hereditary and congenital alopecia and hypotrichosis; Defects of the hair shaft; Hypertrichosis; Traumatic alopecia; Alopecia areata; Cicatricial alopecia; Hair colour; Infections and infestations of the hair and scalp; Psychological factors and disorders of the hair; Hair cosmetics; Hair and scalp in systemic diseases; Diseases of the scalp and skin diseases involving the scalp; Naevi, tumours and cysts of the scalp.

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3675

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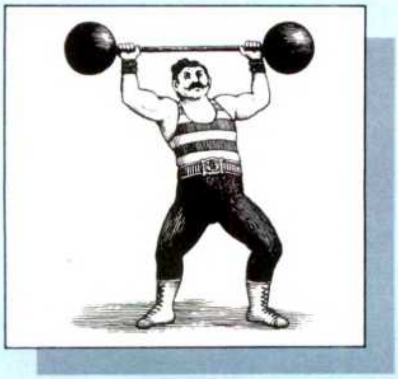

Sterk werkend

Benadert werkingsniveau 4 in de vasoconstrictietest[1]. Het therapeutische effect treedt snel in[2].

1. Intern Rapport, Medische Afdeling, Boehringer Ingelheim B.V.
2. Cahn, M.M. et al. Curr. Therap. Res. (1977) 2, 297.

Juiste balans

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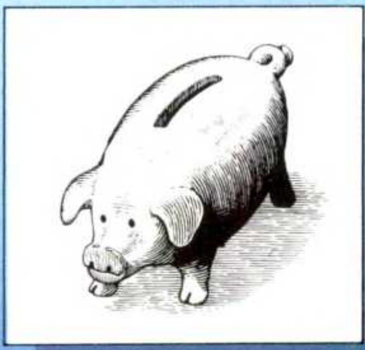
3. Turi, J.S. et al. J. Pharmac. Sci. (1979) 3, 275.



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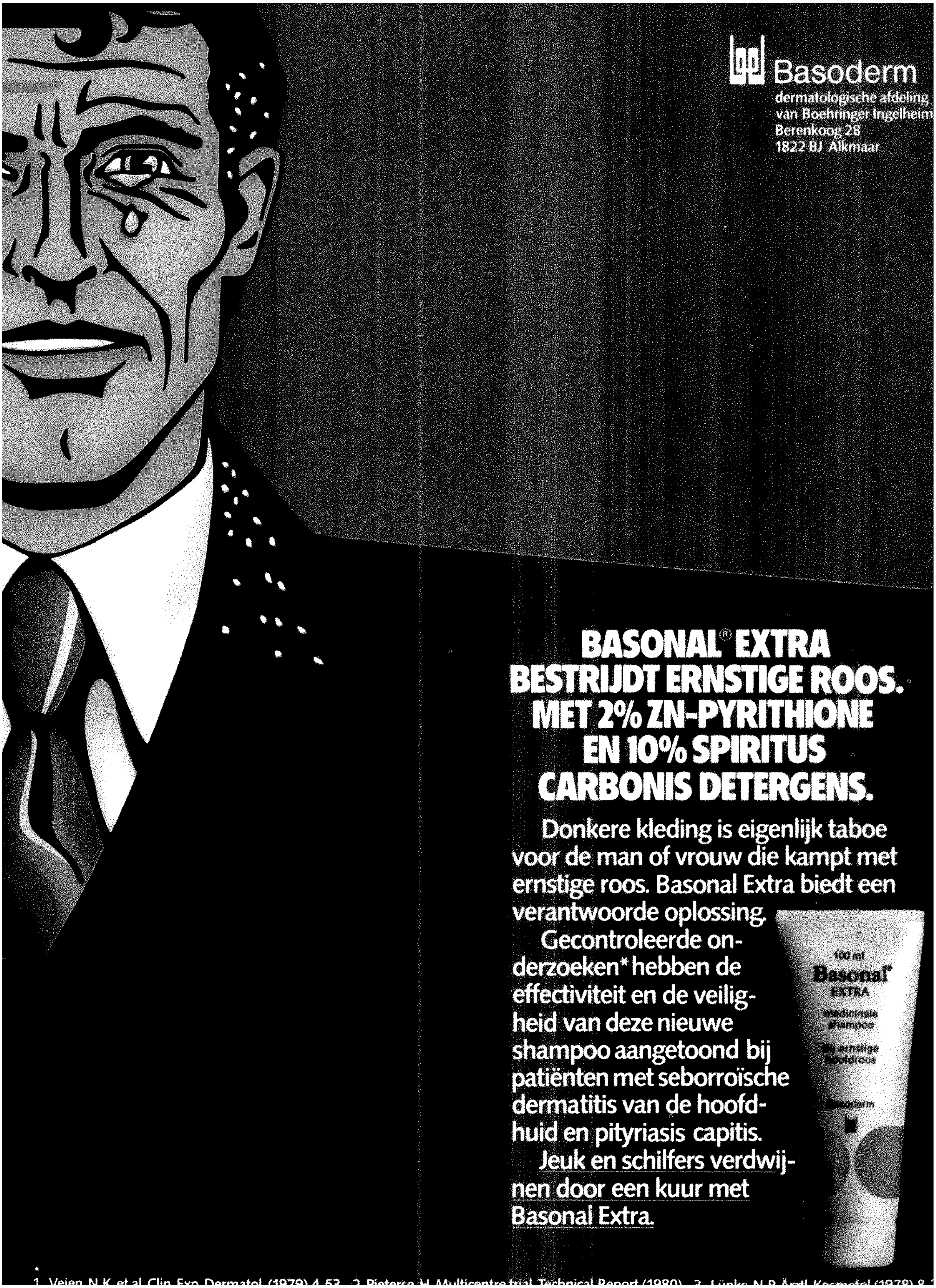

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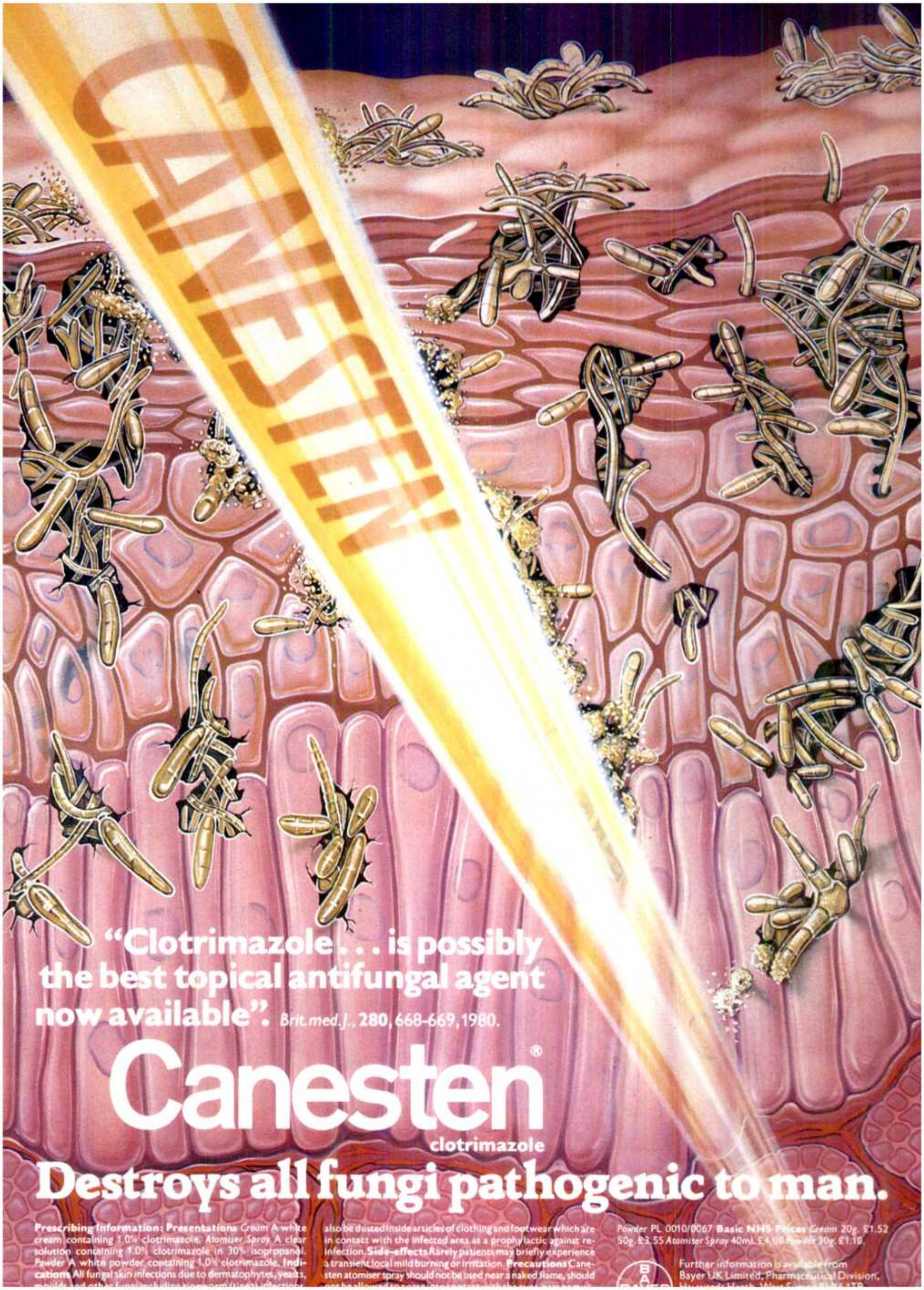
* 1. Veien N.K. et al. Clin. Exp. Dermatol. (1979) 4, 53. 2. Pieterse H. Multicentre trial. Technical Report (1980). 3. Lünke N.P. Ärztl. Kosmetol. (1978) 8,



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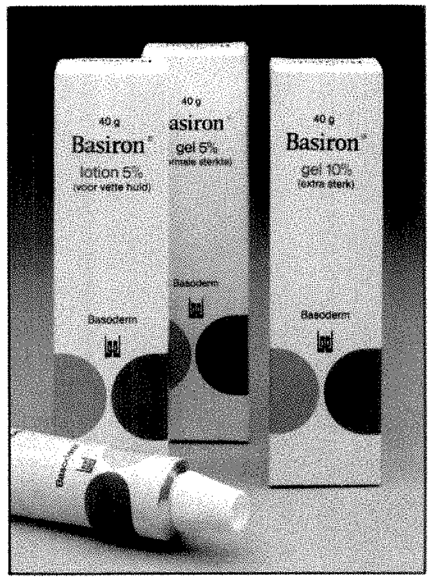

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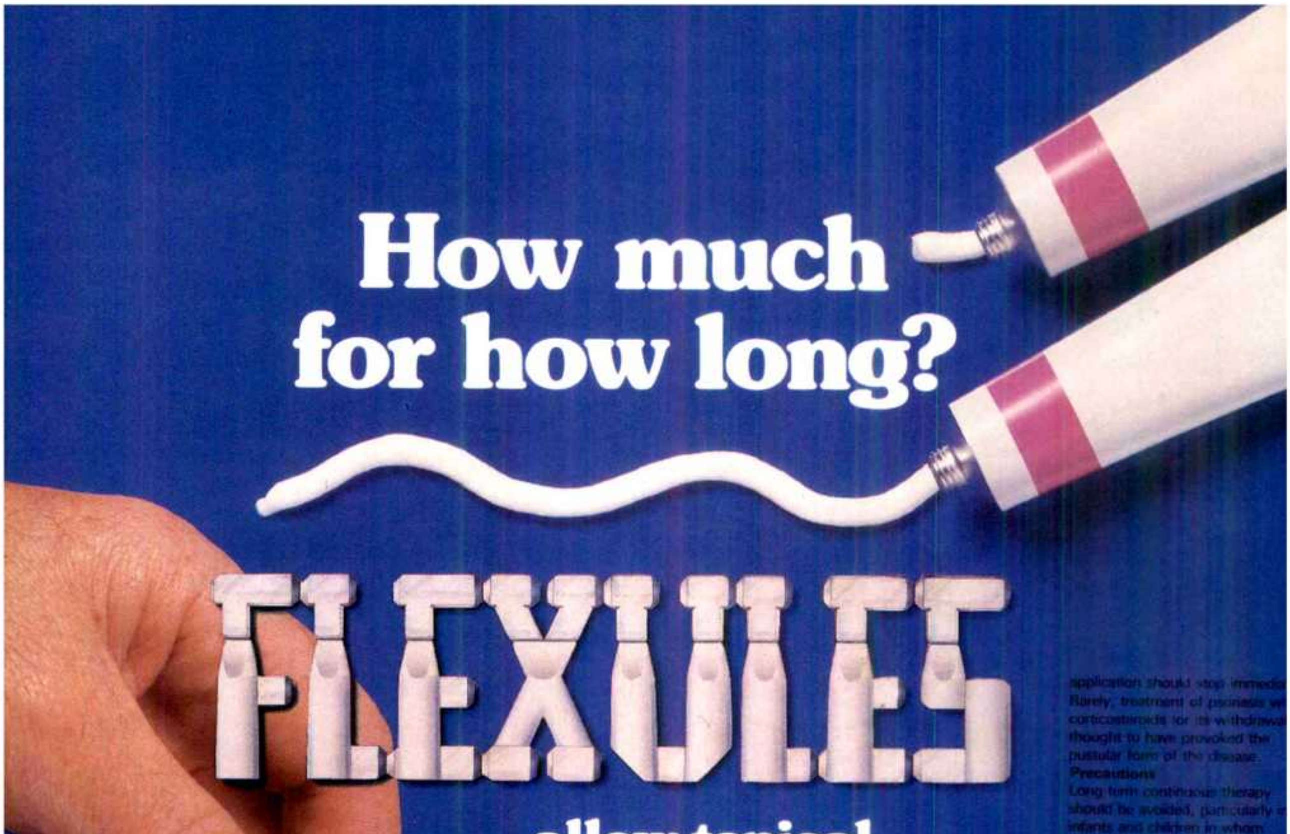

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With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

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prolonged treatment with potent
topical corticosteroids.
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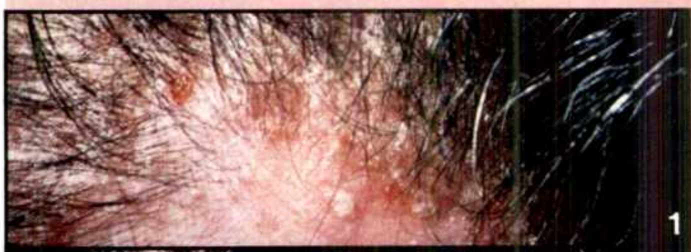
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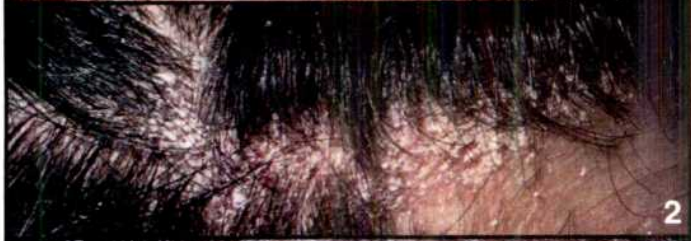
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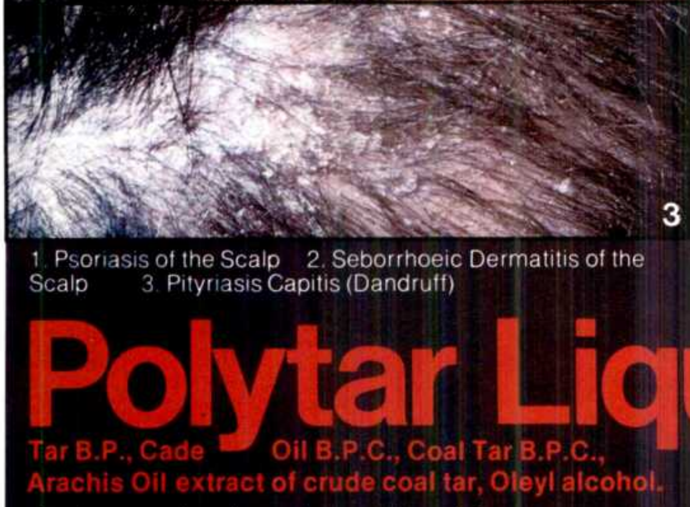
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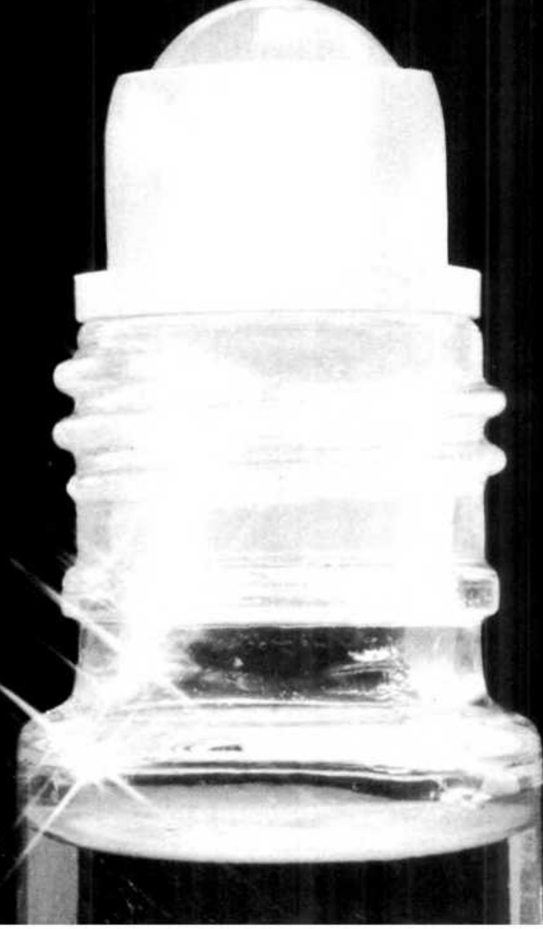

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Clinical and Laboratory Investigations

T cell subsets and Langerhans cells in lichen planus: *in situ* characterization using monoclonal antibodies

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SUMMARY

Skin biopsies from four patients with lichen planus were studied using monoclonal antibodies directed against T lymphocytes. Anti-T1 and anti-T3 antibodies, which react with all peripheral T cells, stained most cells in the dermal infiltrates. The majority of infiltrating cells also stained with anti-T4 and anti-T4b antibodies, which react with helper/inducer cells, whereas a minority of cells stained with anti-T8 antibody, which reacts with cytotoxic/suppressor cells. Surface IgM was not identified on any infiltrating cells, providing evidence against B cell participation. Intraepidermal and dermal cells with long cytoplasmic extensions stained with anti-T6 antibody in all cases, defining them as Langerhans cells or their precursors. T6-positive cells were seen in greater number than in normal control epidermis and dermis. The results indicate that well-developed lesions of lichen planus are characterized by an influx of helper/inducer T lymphocytes and increased numbers of Langerhans cells. These observations support the contention that cellular immunity is important in the pathogenesis of this disorder.

The pathogenesis of lichen planus is unknown. While the initial event in lesion development is obscure, considerable evidence implicates both humoral and cell-mediated immunity in the formation of lesions. Globular deposits (Civatte bodies) containing immunoglobulins, principally IgM, complement and fibrin are present in the dermis in 85–100% of cases. Granular deposits of immunoglobulin and complement have also been observed at the dermo-epidermal junction and in foci of inflammatory cells (Harrist & Mihm, 1979). Because immunoglobulin and complement deposition in lichen planus is limited primarily to Civatte bodies, which are apoptotic keratinocytes (Weedon, 1974), it appears that humoral events are most likely to be secondary.

The clinical and histological findings in certain cases of graft-vs-host disease are virtually identical to those of lichen planus (Hashimoto, 1976) implying a role for cellular immunity in

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lichen planus. Indeed, investigators, using a variety of techniques, have shown the lichenoid infiltrate in lichen planus to be composed primarily of T lymphocytes (Walker, 1976; Alario *et al.*, 1978; Bjerke & Krogh, 1978; MacDonald *et al.*, 1978; Dockrell & Greenspan, 1979). To define further the T cell infiltrate and to characterize Langerhans cell participation in lichen planus, we examined the cell populations in well-developed lesions, with monoclonal antibodies directed against T lymphocytes as well as Langerhans cell surface antigens (Reinherz & Schlossman, 1980; Murphy *et al.*, 1981; Fithian *et al.*, 1981).

MATERIALS AND METHODS

Processing of tissue

A 4 mm punch biopsy specimen was taken from a single lesion of lichen planus in four patients. For controls, a single 4 mm punch biopsy of normal lateral upper arm skin of two healthy adult males was performed. The tissue was immediately bisected, with one half placed in 10% buffered formalin for routine histological examination and the other half frozen in OCT Compound (Ames Co., Div. of Miles Laboratory, Inc., Elkhart, Ind.) and stored at −70°C.

Monoclonal antibodies

A series of seven monoclonal antibodies reactive with thymocyte and/or peripheral T lymphocyte surface antigens was used (Reinherz *et al.*, 1979a,b,c; Bhan *et al.*, 1980; Reinherz & Schlossman, 1980; Reinherz *et al.*, 1980; Poppema *et al.*, 1981). Anti-T1 antibody and anti-T3 antibody react with 100% of peripheral E-rosette positive T cells and approximately 10% of thymocytes. Anti-T6 antibody reacts with 70% of thymocytes but not with peripheral T cells. This antibody has recently been shown to react also with Langerhans cells in the skin (Murphy *et al.*, 1981; Fithian *et al.*, 1981). Anti-T4 antibody, which detects the helper/inducer T cell subset, reacts with 60% of peripheral T cells, while anti-T8 antibody, which detects suppressor/cytotoxic T cells, reacts with 20–30% of peripheral T cells. Another monoclonal antibody termed anti-T4b (clone SFCI-12T4D11, provided by Dr Ellis Reinherz, Sidney Farber Cancer Institute, Boston, Massachusetts), which shows reactivity similar to that of anti-T4 antibody in both cell suspensions and tissue sections (Reinherz & Bhan, unpublished observations), was used as well.

In addition, a monoclonal antibody (anti-I1) reactive with a non-polymorphic region of human Ia-like antigens (Reinherz *et al.*, 1979d) and a monoclonal antibody reactive with human IgM (provided by Dr L. Nadler, Sidney Farber Cancer Institute, Boston, Massachusetts), were used.

Immunoperoxidase studies

Four micron-thick frozen sections were air-dried, fixed in acetone for 5 min and stained by a four-step peroxidase-anti-peroxidase (PAP) method (Bhan *et al.*, 1980; Poppema *et al.*, 1981). Sections were first incubated with a 1:500 dilution of mouse ascites containing one of the monoclonal antibodies for 60 min at room temperature followed by 30 min incubations with rabbit anti-mouse Ig (Dakopatts A/S, Copenhagen, Denmark), swine anti-rabbit Ig, and lastly with peroxidase-rabbit-antiperoxidase reagent (Dakopatts A/S, Copenhagen, Denmark). Between each incubation, sections were washed in three changes of phosphate-buffered saline, pH 7·4.

Staining was achieved by incubation of sections in an acetate buffer solution (pH 5·0) that contained 3-amino-9-ethylcarbazol (Aldrich Chemical Co., Inc., Milwaukee, Wis.), dimethylformamide and hydrogen peroxide. The sections were washed in acetate buffer and mounted in Elvanol (E.L. Dupont de Nemours & Co., Wilmington, Del.).

RESULTS

Light microscopic and direct immunofluorescence examination of the tissue confirmed the clinical impression of lichen planus. Each of the four specimens revealed the characteristic histological changes of lichen planus and direct immunofluorescence revealed moderate to large numbers of colloid bodies which fluoresced after application of anti-human IgM, IgG, C3 and fibrin. Granular immune reactant deposition was not identified at the dermo-epidermal junction.

The vast majority of infiltrating cells stained with anti-T1, anti-T3, anti-T4 and anti-T4b antibodies, whereas only a minority of cells reacted with anti-T8 antibody (Figs 1 and 2). The staining achieved with anti-T1, anti-T3 and anti-T4 antibodies was weak, whereas intense staining was seen with anti-T4b (Fig. 1). In one case, significant epidermal alteration associated with a slight cellular infiltrate and papillary dermal fibrosis suggested that the lesion was in a phase of resolution. $T8^+$ cells formed an appreciable proportion of the T cell population.

There was no staining of cells with antibody to IgM, indicating an absence of IgM^+ B cells in the infiltrate.

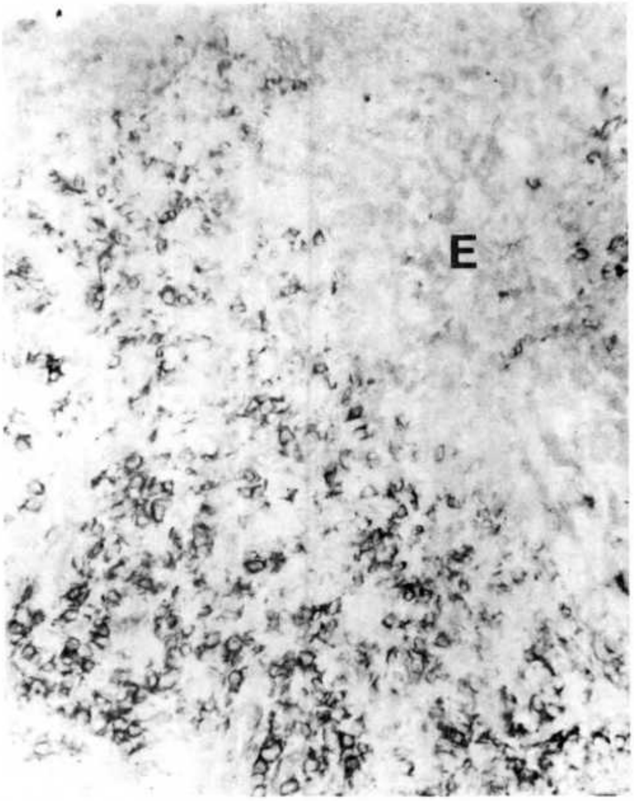

FIGURE. 1. Frozen tissue section of a lesion of lichen planus. A large number of $T4^+$ cells are observed in the papillary dermis, some in close contact with the epidermis (E) (× 256).

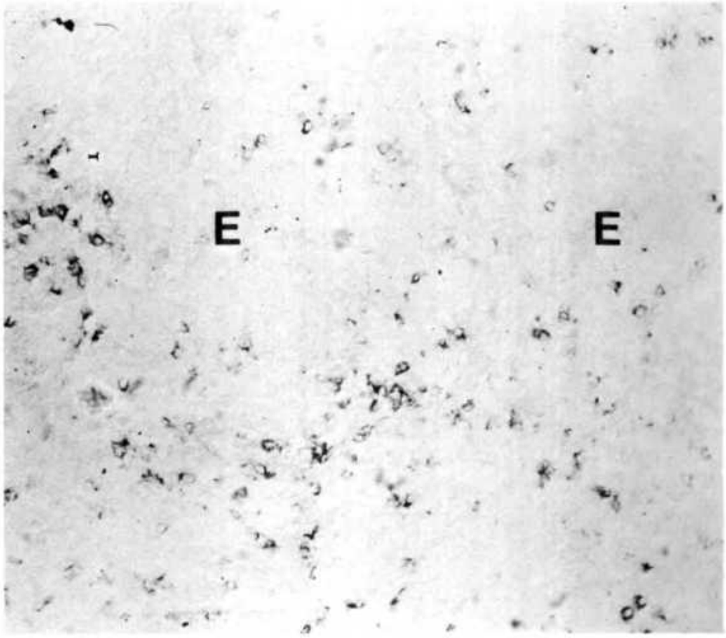

FIGURE 2. Scattered T8^{+} cells are present in the papillary dermis and adjacent to the epidermis (E) in a lesion of lichen planus (× 256).

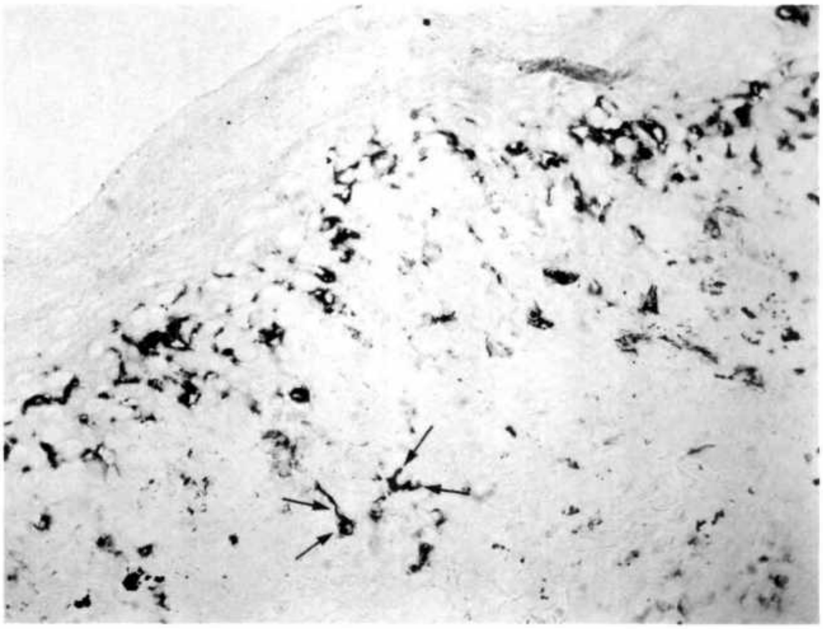

FIGURE 3. A lesion of lichen planus shows a large number of dendritic cells (Langerhans cells) stained with anti-T6 antibody in the epidermis. Similar cells are present in the papillary dermis (arrows) (× 256).

Throughout the epidermis were large dendritic cells which stained with anti-T6 monoclonal antibody (Fig. 3). Morphologically similar, less numerous cells also stained with anti-Il (anti-Ia) antibody. The T6$^+$ cells were considered to be Langerhans cells. In the dermal infiltrates, similar, although fewer, dendritic cells were also T6$^+$. A small number of other mononuclear cells in the infiltrate and endothelial cells also stained with anti-Ia antibody.

In the two control specimens, only rare dermal T8$^+$ cells were identified. Within the epidermis, T6$^+$ dendritic cells were observed; however, they were much fewer in number than those seen in the lesions of lichen planus.

DISCUSSION

A series of monoclonal antibodies were employed to study the nature of the cellular infiltrate in the frozen tissue sections of lichen planus by the immunoperoxidase technique. A majority of the cells in the infiltrate reacted with anti-T1, anti-T3, and anti-T4 antibodies. The present study confirms the T cell nature of the infiltrate in lichen planus, as suggested by previous studies employing E-rosetting techniques or heteroantisera against T cells (Alario *et al.*, 1978; MacDonald *et al.*, 1978). More importantly, most of the T cells in the infiltrate are of the helper/inducer subset. In contrast, only a small fraction of cells in the infiltrate stained with anti-T8, which reacts with suppressor/cytotoxic T cells. In one presumably resolving lesion, T8-positive cells formed an appreciable proportion of the cellular infiltrate indicating that dynamic alterations of the infiltrate occur during the course of these lesions.

The absence of plasma cells in the infiltrate and lack of staining for B-cells in the present study indicate that the immunoglobulins deposited in lichen planus lesions are derived from plasma proteins and are not locally produced.

As compared to normal skin, increased numbers of large dendritic cells in the epidermis and in the dermis stained with anti-T6 antibody and to a lesser extent, with anti-Ia antibody. Anti-T6 antibody reacts with a majority of cortical thymocytes but not with mature peripheral T cells (Reinherz & Schlossman, 1980; Bhan *et al.*, 1980). In a recent study (Poppema *et al.*, 1981), large cells staining with anti-T6 antibody were found in the paracortex of certain lymph nodes, including a case of dermatopathic lymphadenitis. In addition, T6$^+$ cells in the normal epidermis appear to be Langerhans cells, as shown by their dendritic nature and the presence of Ia-antigens. More recently, employing immunoperoxidase electron microscopy, we have demonstrated Birbeck granules in the cytoplasm of T6$^+$ cells in the epidermis (Murphy *et al.*, 1981), establishing T6 antigen as a new immunological marker for epidermal Langerhans cells. Additional evidence of the association of T6 antigen with Langerhans cells using double-labelling techniques has recently been reported (Fithian *et al.*, 1981). Dermal dendritic T6$^+$ cells were also observed in lichen planus. These cells may represent either so-called 'indeterminate' (Langerhans precursor) cells or dermal Langerhans cells.

Langerhans cells, which have some characteristics of mononuclear phagocytes, are the antigen-presenting cells in allergic contact reactions (Thorbecke, Silberberg-Sinakin & Flotte, 1980). The presence of a large number of Langerhans cells associated with numerous T4$^+$ cells adjacent to the epidermis as well as the absence of B cells, strongly suggests that cell-mediated immunity is important in the pathogenesis of lichen planus. The development of lichen planus-like lesions in some cases of chronic graft-vs-host disease would also support this contention (Saurat & Gluckman, 1977).

In lichen planus, the basal keratinocytes appear to be the primary site of immunological injury. Alterations in basal keratinocyte membranes in lichen planus have been suggested by diminished lectin binding to these cells in contrast to supra-basal cells, which have normal binding (Holmstrup & Dabelsteen, 1979). Thus, lichen planus lesions may represent a T cell ($T4^+$ helper/inducer cell) mediated response directed against altered basal keratinocytes. Furthermore, Langerhans cells may play an important role in the processing and presentation of antigen to $T4^+$ helper/inducer cells in this disorder.

ACKNOWLEDGMENTS

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The growth of vibrissa dermal papilla cells *in vitro*

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SUMMARY

Vibrissa dermal papilla cells have been successfully grown and serially cultured and their early behaviour compared with cultured feather dermal papillae. Initially the vibrissa cells demonstrated singular characteristics, including poor spreading ability, before eventually forming a fibroblast-like population of cells.

Comparatively few studies have exploited the adult hair follicle as a source of cell culture material. While cultures have been established by complete disruption of lower-hair bulbs (Frater, 1975) and from mildly trypsinized explants (Uzuke, Takeshita & Morikawa, 1975), both methods produce a mixed population of cells, whose exact intrafollicular site of origin is unknown.

The large size of the vibrissa follicle makes it amenable to microsurgical manipulation of discrete follicular components, and *in vivo* studies have shown the dermal papilla to be necessary for both the induction and maintenance of hair growth (Oliver, 1966, 1967). This study investigated the possibility of growing a population of dermal papilla cells in culture and the observations constitute the first description of these important and highly specialized cells *in vitro*.

MATERIALS AND METHODS

Extraction of papillae

Dermal papillae were dissected out from the major vibrissa follicles of inbred PVGC hooded rats (colony, Dundee University). Animals were killed and the whisker follicles exposed as previously described (Oliver, 1966). The lower or proximal halves of the follicles were then removed and placed in sterile 199 medium (Gibco). The papillae were extracted using iridectomy scissors and watchmaker's forceps and carefully cleaned of adherent epidermal components (Fig. 1). For comparative studies feather papillae were dissected from follicles containing newly emerged feathers on White Leghorn hens. All microsurgical procedures were carried out under a binocular microscope (× 20).

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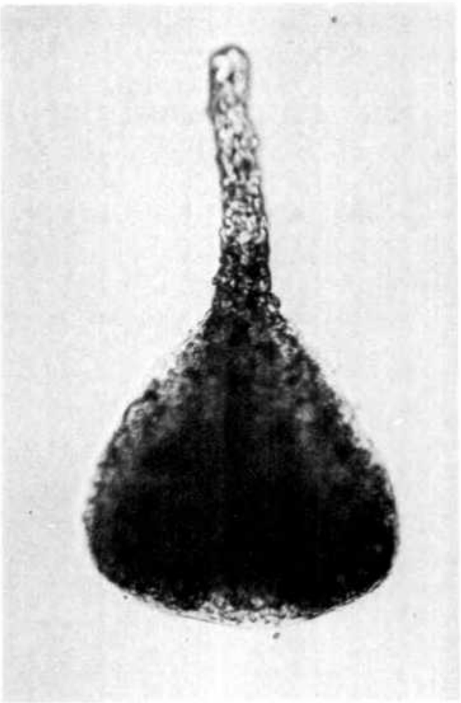

FIGURE 1. Intact vibrissa dermal papilla (× 130).

Culture methods

Explanted papillae were initially grown on the glass coverslips covering plastic Cruickshank culture chambers (Sterilin) at 37°C. Just prior to their introduction the papillae were subjected to slight physical disruption as this was found to result in both a greater likelihood of attachment and earlier cellular activity. The standard culture medium consisted of Eagles MEM containing penicillin (50 iu/ml) and streptomycin (50 μg/ml) supplemented with 15–20% fetal calf serum. All tissue culture materials were obtained from Gibco. Observational studies were carried out on over fifty chambers containing either single or multiple papilla explants. Cell outgrowths were subcultured using 0·25% trypsin in phosphate-buffered saline/EDTA (0·2 mg/ml). Establishment of a large primary cell population was achieved by initial subculturing onto 35 mm plastic petri dishes (Sterilin) in an atmosphere of 5% CO_2 in air followed by transfer to 25 cm^2 plastic flasks (Nunc).

RESULTS

After approximately three days in culture, cells detached from the explants and settled with no distinctive configuration in the immediate vicinity of the papilla (Fig. 2). At this stage they appeared irregularly shaped, and often very granular, especially in the perinuclear area. Where the break up of the papilla proceeded rapidly, a central sheet of closely packed cells resembling transformed fibroblasts was formed. During this phase of establishment cell division was only occasionally observed. This initial behaviour contrasted with parallel observations of the feather papilla explants. At the same stage these cells had streamed rapidly outwards, immediately assuming a typical fibroblast-like organization and appearance (Fig. 3).

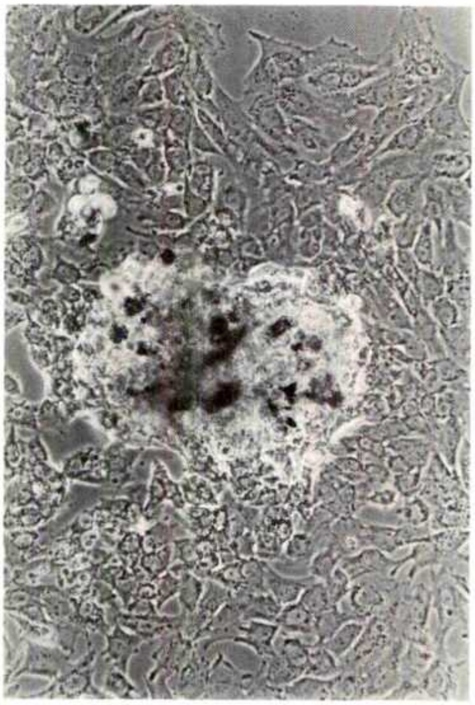

FIGURE 2. Five-day old vibrissa papilla culture with cells settling in the vicinity of the explant (× 170 phase contrast).

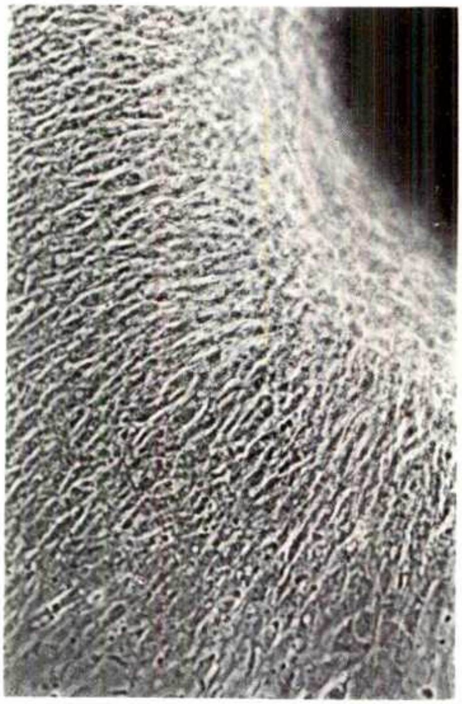

FIGURE 3. Five-day old feather papilla culture showing cells streaming from the explant (× 170 phase contrast).

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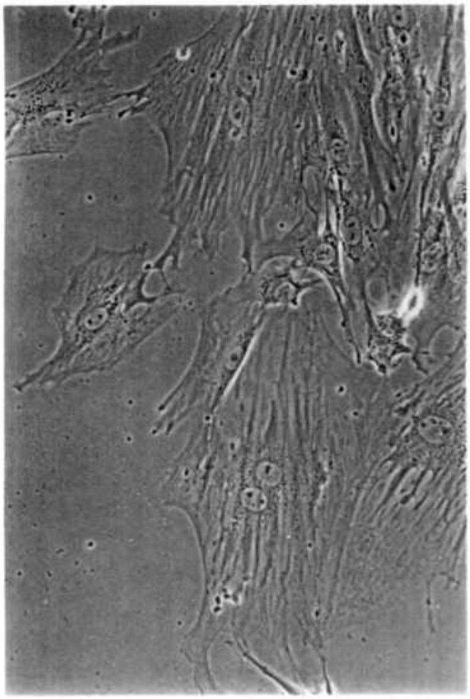

FIGURE 4. Ten-day old vibrissa culture. Flattened cells on periphery of the outgrowth (× 170 phase contrast).

As vibrissa cells began to spread outwards they assumed a more fibroblast-like organization forming a monolayer of spindle shaped cells in parallel arrays. Towards the periphery of the outgrowth, cells became larger and flattened with a variety of irregular shapes (Fig. 4). Characteristically these exhibited numerous cytoplasmic processes and contained phase-dark microfilament bundles giving them a striated appearance. An individual colony size of 3 to 4 mm diameter was attained after 2–3 weeks in culture after which spreading was gradual. With continuing division cells were seen to overlap in the middle of the outgrowth. This often resulted in the formation of rounded clumps of cells which eventually necrosed and became indistinguishable from the residual material remaining from the original papilla explant. In other cultures thickening took the form of wider sheets of cells. In both cases the dense area became visible to the naked eye as opaque cell masses in the chambers.

On being reseeded in single cell suspension papilla cells formed a fibroblast-like population when grown in petri dishes. Cell numbers were then sequentially built up until flask cultures were established. The cells have been successfully maintained in flask cultures through 16 passages over a period of 12 weeks.

DISCUSSION

The present study has demonstrated that adult dermal papilla cells are capable of proliferation under normal conditions *in vitro* eventually producing a fibroblast-like population of cells. Normally, from an early stage of development *in vivo*, hair papilla cells display a notable absence of proliferation (Wessells & Roessner, 1965) and this characteristic persists in adult follicles (Pierard & de la Brassine, 1975).

Compared with feather papilla explants the vibrissa papilla cells consistently demonstrated poor spreading ability and an apparent initial reluctance to divide. This behaviour makes an interesting parallel with some of the properties of the papilla *in vivo*. For example, the cells of the papilla are known to possess numerous intercellular attachments both in human hair follicles (Hashimoto & Shibazaki, 1975) and rat vibrissa follicles (unpublished observation) and this probably contributes to the inherent stability of the papilla. This stability is also demonstrated by transplanted papillae which maintain their morphological integrity and can be located as discrete balls of cells for months post-operatively (Oliver, 1970).

This successful growth of hair papilla cells in culture now makes them available for study in relation to their role in the growth of hair.

Using microsurgical techniques epidermal components can also be isolated from the vibrissa follicle. Further investigation could include growing follicular epithelial cells both in isolation and in controlled interaction with papilla cells in culture. The feasibility of this idea is enhanced by a report describing the culture of epithelial cells from human hair follicles (Weterings, Vermorken & Bloemendal, 1981).

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An assessment of the effect of photochemotherapy (PUVA) and UV-B phototherapy in the treatment of psoriasis

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SUMMARY

We have shown that it is possible to produce a satisfactory clinical response with a low UV-A dosage regime in approximately one-third of patients with widespread chronic psoriasis. UV-B phototherapy is as effective initially, but the improvement is not as well maintained as that resulting from photochemotherapy (PUVA). However, in view of the probability that unwanted side effects of PUVA are related to the amount of UV-A irradiation administered, it would seem appropriate to restrict the use of the higher dosage regimes to selected patients and to those who have not responded to an initial low dosage regime or to UV-B phototherapy.

Photochemotherapy (PUVA) is now established as an important component in the treatment of psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1975a, b; Swanbeck *et al.*, 1975; Wolff *et al.*, 1976; Melski *et al.*, 1977; Vella Briffa *et al.*, 1978; Siddiqui & Cormane, 1979; Cripps & Lowe, 1979; Roenigk *et al.*, 1979; Epstein & Farber, 1980). Although producing no serious short-term side effects, there is still concern regarding the possibility of longer-term sequelae. Repeated courses of PUVA may be required throughout the lifetime of an individual and since chronic side effects are likely to be dose-related it is important to determine the minimal dosage regime which will produce acceptable clinical improvement.

The larger reported studies have used relatively high intensity regimes with mean total UV-A doses ranging from 50–260 J/cm^2 (Wolff *et al.*, 1976; Melski *et al.*, 1977; Vella Briffa *et al.*, 1978; Roenigk *et al.*, 1979). For example, in a multi-centre trial involving 1,309 subjects satisfactory clinical results were achieved with a mean total dose of 191 J/cm^2 in those subjects with skin type I; with skin type VI the mean total dose reached the exceptional level of 469 J/cm^2 (Melski *et al.*, 1977). In another co-operative study involving sixteen centres, clearance was obtained in 85% of the subjects after a mean of 186 J/cm^2 with total exposure doses ranging from 15·7–1002·5 J/cm^2 (Roenigk *et al.*, 1979). Taking an overall view of these conventional 'high' intensity

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irradiation dosage regimes, it would appear that the average dose administered to achieve a satisfactory clinical result was of the order of 100 J/cm^2 (Parrish *et al.*, 1974; Vella Briffa *et al.*, 1978; Roenigk *et al.*, 1979).

We have previously reported that an acceptable clinical improvement was obtained in thirty-four out of fifty-six psoriatic subjects using low intensity irradiation which produced its effect with a mean total dose of 40 J/cm^2 (range 18–80 J/cm^2) (Lakshmipathi *et al.*, 1977). It was decided therefore to (1) assess the results achieved by such a low intensity irradiation regimen in comparison with those obtained by a more conventional high intensity regimen, (2) investigate an earlier impression that those patients who did not respond to low intensity treatment would do so when transferred to the higher intensity regimen, (3) compare the results of treatment by UV-B phototherapy with those obtained by photochemotherapy, and (4) determine whether UV-B phototherapy would control relapses following cessation of photochemotherapy.

MATERIALS AND METHODS

Photochemotherapy

During the period December 1978 to March 1980, sixty-two subjects (thirty-eight males and twenty-four females, mean age 38 years, range 19–70 years) with extensive psoriasis were treated. The psoriasis was mainly of the chronic plaque variety with guttate lesions and flexural involvement in some instances. During photochemotherapy treatment was otherwise limited to the application of emulsifying ointment or yellow soft paraffin.

Oral 8-methoxypsoralen (8-MOP) (Meladinine 10 mg tablets, Basotherm GmbH) in a dose of 0·6 mg/kg body weight was taken 2 h prior to UV-radiation. Plasma 8-MOP levels were estimated 2 h after ingestion on several occasions in thirty subjects. The low intensity UV-A irradiation was provided by means of two cubicles. Cubicle 1 was rectangular and contained horizontal arrays of 2-ft and 4-ft UV-A fluorescent tubes (Thorn/Atlas, Ultraviolet, 20 W and 40 W) which produced an exposure dose of 1·5 J/cm^2 at an average skin surface distance of 250 mm in 20 min. Cubicle 2 was square, containing vertically mounted 5-ft UV-A fluorescent tubes (Thorn Ultraviolet 65/80 W) giving a 20-min exposure dose of 2·4 J/cm^2. The high intensity irradiation was provided by a Waldmann PUVA 6000 (cubicle no. 3) capable of delivering 8 J/cm^2 in the same 20 min period. The fall in intensity with ageing of the tubes was monitored and adjustments made in the duration of treatment to maintain uniformity of UV-A dosage. Irradiance measurements were made with a standard Waldmann PUVA meter. Forty subjects were randomly allocated to the low irradiation regime and twenty-four to the high intensity irradiation programme. The minimal phototoxic dose (MPD) was determined and the skin type recorded, and the subsequent programme based on this information with the starting dose taken as 0·5 J/cm^2 less than the MPD (Wolff *et al.*, 1977a).

Treatment was given twice weekly and increments of 0·5 J/cm^2 or exceptionally of 1 J/cm^2 were added until such time as rapid clinical improvement was obtained within the selected maximum output at 20 min of irradiation. The frequency of treatment was reduced to weekly and later fortnightly within a range of 4–8 weeks after obtaining this initial response and subsequently stopped in all cases. Regular assessment of the effect of the treatment was made at weekly intervals and a monthly assessment was made by one of us who had not been involved in the regular day-to-day observation. The criteria used to determine grades of improvement were as follows: satisfactory—clear with or without minimal residual activity or improving rapidly at all affected sites; moderately satisfactory—subjectively satisfactory but less dramatic than satisfactory; unsatisfactory—slowly improving, stationary, or worse.

UV-B phototherapy

Twenty-five subjects with psoriasis of severity similar to those treated with photochemotherapy were selected for treatment with UV-B phototherapy. In addition, thirty-seven subjects who had been previously treated by photochemotherapy and had relapsed at variable intervals after the photochemotherapy had been discontinued were also treated with UV-B phototherapy.

The UV-A sources in cubicles 1 and 2 were replaced by UV-B fluorescent tubes. In the first instance Westinghouse FS-20 and FS-40 'Sunlamp' tubes were used with an emission from approximately 275–340 nm, peaking around 312 nm. The irradiance was 0·5 mW/cm^2 and the average minimal erythema dose (MED) was 4 min. The method of therapy detailed below requires incremental increases in exposure at each successive treatment. We have found that a 20-min exposure is maximal for most patients. This maximal exposure time was reached early in the course of treatment with the Westinghouse tubes so that required increases were not possible. The lamps were therefore replaced with Philips Colour 12 (TL20W12 and TL40W12) fluorescent tubes and the number of tubes was increased. The irradiance obtained was increased by a factor of 3 to 1·5 mW/cm^2 but the average MED was decreased by more than a factor of 3 because, although the emission spectra of the two lamp types are similar, that of the Philips Colour 12 has a greater proportion of energy in the erythemogenic wavelength region below 310 nm (Mackenzie & Johnson, 1981). The irradiance values are estimates of the total, unweighted UV-irradiance derived from measurements with a filtered UV detector (Jagger, 1967) and based upon a comparison of this detector response with that of a standard thermopile.

The MED was determined for each patient and treatment started at 70% of this MED. The aim was to produce a mild erythema response with each subsequent dose. To this end, the subsequent dose was increased by 40% if there was no erythema, by 20% if slight erythema, and the previous dose was repeated if more than slight erythema ensued. If a patient produced a more severe 'sunburn' reaction the subsequent three dosages after this had settled were calculated as 50%, 75% and 100% of that producing the reaction. Patients were advised to avoid additional solar exposure. Once a satisfactory improvement had been obtained, the frequency of treatment was reduced to once weekly and subsequently stopped.

RESULTS

Photochemotherapy

Low intensity irradiation. Sixteen of the forty subjects originally allocated to the low intensity irradiation regimen were retained under this regimen until a definitive assessment at 20 weeks after the initiation of treatment. One subject was lost to the study and twenty-three who failed to respond were transferred to the high intensity regimen.

Fifteen of the sixteen had achieved a satisfactory response after a mean of 7·9 weeks (range 4–19 weeks) of treatment and a mean total exposure dose of 21·3 J/cm^2 (range 9–92 J/cm^2). At the 20-week assessment, fourteen were classified as having had a satisfactory response to treatment, one as moderately satisfactory and one as unsatisfactory. By the 20th week, ten had already stopped treatment (duration of treatment ranging from 6–16 weeks) and the remaining six were receiving weekly or fortnightly maintenance. Eight of the sixteen remained in satisfactory remission for more than 30 weeks (mean remission time for the fourteen satisfactory responders, 31 weeks, range 8–48 weeks) after stopping treatment.

Transfer of non-responders from low to high intensity irradiation. The twenty-three subjects who had failed to respond to the low intensity regimen were transferred to the high intensity treatment at times which varied from 7–20 weeks from the beginning of the study; thirteen of these twenty-three subjects were subsequently classified as having obtained a satisfactory response, seven moderately satisfactory and three unsatisfactory (i.e. an assessment at 20 weeks after the initiation of treatment). Eventually, nineteen of the twenty-three did achieve a satisfactory response within a range of 9–34 weeks (mean 20 weeks) of the combined low and high intensity programmes with total exposure doses which ranged from 37–186 J/cm^2 (mean 112·6 J/cm^2). However, four of these nineteen subjects did not maintain the improvement despite a continued high intensity irradiation treatment. When treatment was eventually stopped, twelve of these nineteen remained in satisfactory remission for more than 12 weeks (mean remission period 11·7 weeks, range 1–26 weeks).

High intensity irradiation. Assessment at 20 weeks showed that, of the twenty-two subjects allocated to the high intensity irradiation regimen, twenty had achieved a satisfactory response and two were moderately satisfactory after a mean total exposure dose of 127·6 J/cm^2 (range 49–260 J/cm^2). By this 20-week stage thirteen of the twenty-two subjects had stopped treatment, six were receiving weekly or fortnightly maintenance doses and three required to continue twice weekly treatment.

Over the whole course of the study, all twenty-two subjects had achieved a satisfactory response after a mean of 8 weeks treatment (range 4–20 weeks) and a mean total exposure dose of 60·6 J/cm^2 (range 6–260 J/cm^2). All had stopped treatment after a mean 26·8 weeks (range 8–68 weeks). The mean duration of remission in this group was 25·5 weeks (range 1–72 weeks) and eleven of the twenty-two subjects remained in satisfactory remission for more than 30 weeks after stopping treatment.

UV-B phototherapy

Subjects treated previously with conventional therapy. Of the twenty-five subjects treated with UV-B phototherapy, twenty had achieved a satisfactory response after a mean of 10·6 weeks treatment (range 3–26 weeks) and a mean total exposure dose of 8·75 J/cm^2 (range 0·7–15·8 J/cm^2). For twenty-three of the twenty-five subjects, treatment was stopped at times ranging from 8 to 43 weeks (mean 22 weeks) and total exposure doses of 0·4–40·9 J/cm^2 (mean 19·3 J/cm^2). At the time when treatment was stopped it was noted that the initial satisfactory response in the twenty subjects had not been maintained in all of them in that now, only sixteen continued to have a satisfactory classification, 3 were moderately satisfactory and 4 were unsatisfactory. Phototherapy was continued in the remaining 2 subjects. Of the 16 satisfactory responders, only 8 remained in remission for more than 8 weeks (mean, 15·2 weeks; range 1–34 weeks).

Subjects who had relapsed after previous photochemotherapy. Of the thirty-seven subjects who were treated with UV-B phototherapy following relapse after stopping photochemotherapy, thirty had achieved a satisfactory response after an average of 12·3 weeks treatment (range 4–30 weeks) and a mean total exposure dose of 8·78 J/cm^2 (range 1·3–42·4 J/cm^2).

Treatment was stopped for twenty-three subjects in this group at times varying from 8 to 39 weeks (mean 23 weeks). At the time when treatment was stopped, it appeared that the initial satisfactory response had once again not been maintained in all of the thirty subjects in that now, only nine would be classified as having a satisfactory response, one was moderately satisfactory and as many as thirteen were considered to be unsatisfactory. The total exposure dose for the twenty-three subjects at the time when treatment was stopped ranged from 5·0–47·5 J/cm^2 (mean 24·4 J/cm^2). Of the nine patients classed as satisfactory on stopping treatment, four remained in remission for more than 7 weeks (mean remission 7·7 weeks, range 1–26 weeks).

The remaining fourteen patients have continued with treatment.

DISCUSSION

Several studies have confirmed the clinical efficacy of photochemotherapy without however attempting to define the minimal amount of irradiation required to produce an acceptable clinical response. Melski *et al.* (1977) reported clearance in 88% of 1,308 subjects after a mean total dose of 260 J/cm^2, some subjects receiving over 1,000 J/cm^2 to achieve clearance. More recently Roenigk *et al.* (1979) reported similar results in a group of 575 subjects, but with the observation that subjects treated by a plateau method, i.e. limiting any further increase in exposure dose once a steady state of improvement had been achieved, required a lower total exposure dose, lower final dose at clearing and a lower number of treatments to achieve a satisfactory result. Nonetheless, the mean total exposure dose in this group was 185 J/cm^2 and ranged from 15·7 to 1,002·5 J/cm^2. Similarly, by limiting increments in exposure once a steady state of improvement had been achieved, Vella Briffa *et al.* (1978) and Wolff *et al.* (1976) reported as good results while using mean total dosages of the order of 100 J/cm^2.

With any of these treatment schedules given over a period of 2–3 years, premature ageing and premalignant changes typical of chronically sunburned skin, are rarely seen. Sub-epidermal homogenization, disruption of elastic fibres but no elastosis (Wolff *et al.*, 1977b; Bergfeld, 1977), a perivascular coating of a homogeneous material (Hashimoto *et al.*, 1978), acid mucopolysaccharide deposition (Bergfeld, 1977) and rarely amyloid deposition (Greene & Cox, 1979) may occur in the dermis where bizarre giant cells, probably of fibroblast origin, may be seen to persist (Omar, Weismann & Krebs, 1977; Hashimoto *et al.*, 1978). In most cases increased melanocyte activity reverts to normal on maintenance or cessation of treatment (Zaynoun *et al.*, 1977). Patches of epidermal dystrophy, persisting for at least a year, may occur in the non-lesional skin of some patients during PUVA induced clearing (Cox & Abel, 1979), and both nuclear and general cellular irregularities in a disturbed epidermal architecture were observed in sections from more than 40% of biopsies from 243 patients studied retrospectively following a cumulative total exposure dose of 580 J/cm^2 (Gschnait *et al.*, 1980). Whether these epidermal changes are derived from particularly vigorous PUVA therapy, from over-exposure to the UV-B component of the radiation, or from some cause specific to the individual patient, the histopathology has renewed interest in the implications of the photosensitized interactions of 8-MOP with chromosomal DNA.

It is generally accepted that the biologic effects of 8-MOP and UV-A exposure are mediated through changes in DNA (Scott, Pathak & Mohn, 1976). These changes may result in cell death, the inhibition of cellular proliferation and mutagenesis which has been demonstrated in micro-organisms and mammalian cells in culture (Igali *et al.*, 1970; Arlett, 1973; Burger & Simons, 1979; Arlett *et al.*, 1980). The potential for 8-MOP photosensitized mutagenesis in human cells is illustrated by the demonstration of increased sister chromatid exchanges in leucocytes exposed *in vitro* (Carter, Wolff & Schnedl, 1976; Mourelatos, Faed & Johnson, 1976; Mourelatos *et al.*, 1977; Lambert *et al.*, 1978). In addition, chronic exposure to 8-MOP and

UV-A results in skin cancer in experimental animals (Griffin, 1959; Forbes, Davies & Urbach, 1976; Grube, Ley & Fry, 1977).

Considerations of 8-MOP concentration and UV-A exposure dose levels required for the results of the experimental studies in relation to those to which epidermal and dermal cell populations are exposed during PUVA therapy would suggest that there is a potential for somatic mutation, malignant transformation and carcinogenesis in this treatment. However, the relevance of these experimental studies for the situation obtaining in PUVA treated psoriatic skin remains unclear. Although Kraemer & Weinstein (1977) reported a decrease in DNA synthesis in non-stimulated lymphocytes from PUVA treated patients and several authors have found a decrease in mitogen response in lymphocytes taken at various times during a course of PUVA therapy (Lischka *et al.*, 1977; Morison *et al.*, 1979b; Friedmann & Rogers, 1980), others have failed to detect any change (Ortonne *et al.*, 1978; Wassilew, 1978; Fraki, Eskola & Hopsu-Havu, 1979; Harper *et al.*, 1979), and also there is no increase in sister chromatid exchange rate in circulating blood cells from patients undergoing PUVA therapy (Wolff-Schreiner *et al.*, 1977; Faed *et al.*, 1980).

Where skin malignancy has arisen in PUVA treated subjects, there appears to have been a predisposing factor which may be genetic, as in the case of xeroderma pigmentosum (Reed, 1976, 1977), or related to previous treatment with ionizing radiation (Stern *et al.*, 1979) or with arsenic (Brenner *et al.*, 1980), or related to a previous history of malignancy (Stern *et al.*, 1979; Hofmann, Plewig & Braun-Falco, 1979; Verlich, 1979; Tam, van Scott & Urbach, 1979). PUVA treatment may lead to the suppression of cell mediated delayed hypersensitivity reactions (Morison, Woehler & Parrish, 1979a), and the impairment of circulating T cell function detected in some psoriasis patients during photochemotherapy may be an indirect effect of the 8-MOP photosensitization (Haftek *et al.*, 1979). As with UV-B treatment, 8-MOP and UV-A may induce suppressor T cells in mice, leading to the acceptance of strongly antigenic UV-induced tumour cells (Roberts, Schmitt & Daynes, 1979). It is possible then that PUVA therapy may interfere with any immune surveillance which exists in respect of squamous cell carcinomata and that the action of 8-MOP photosensitization in the cases of skin cancer related to PUVA treatment is that of tumour promotion rather than that of a complete carcinogen.

Whatever the exact mechanism, where an increase in skin malignancy is reported to be associated with PUVA, it would appear to be related to high total exposure doses. Moreover, with high intensity irradiation the photosensitized lesions in DNA are less effectively dealt with by error free repair processes (Bridges, 1978). Consideration of these points encourages the use of minimal dose regimens which will produce satisfactory clinical responses. Weismann, Howitz & Boro-Jorgensen (1977) obtained so-called clearance in seventeen out of thirty-one patients with a total of 31 J/cm^2, and almost complete clearance in a further twelve with a total exposure dose of only 53 J/cm^2. Similarly, in a complex study in which both 8-MOP concentration and irradiance were kept at a minimal level, Thune & Volden (1977), using a system in which the irradiance was 2 mW/cm^2, obtained complete healing in thirteen of thirty-one patients, and the response was considered to have been poor in only three. Our previous experience with low intensity photochemotherapy indicated that a number of psoriatics improved satisfactorily with relatively low total exposure doses from 18 to 80 J/cm^2 (Lakshmipathi *et al.*, 1977). This initial impression has been confirmed in the present study; fifteen of forty (37·5%) chronic psoriatics treated with low intensity regimen show as good a degree of improvement in as short a time and with a fraction of the exposure dose as those on a more conventional high intensity irradiation programme (21·3 J/cm^2 compared with 60·6 J/cm^2). In addition, the mean remission time of 31 weeks for the low intensity regimen compared with 25·5 weeks obtained with the high intensity irradiation shows that a satisfactory clinical state is maintained at least as well after this form of treatment.

It is not evident that the patients who failed to respond to low intensity irradiation would necessarily have improved if initially treated with a high intensity regimen. Transfer of these non-responders to the high intensity regimen produced a subsequent satisfactory response in a number of them but, overall, this was achieved less rapidly and to a lesser extent than in either the group which responded well to the low intensity treatment or the group treated from the outset with the high intensity regimen. Duration of satisfactory remission in this group was shorter, mean of 11·7 weeks, than the mean 31 weeks for the low intensity group or the mean 25·5 weeks for the high intensity group.

It is therefore probable that in a larger group of chronic psoriatics, a considerable number, greater than one-third, will benefit rapidly with low intensity irradiation PUVA treatment, requiring a low total exposure dose to achieve an acceptable clinical response. While the majority of subjects initially exposed to high intensity treatment achieve a satisfactory clinical response, the mean total exposure dose required for this response is higher. The transfer of non-responders from the low intensity regimen may be successful in some patients but does not ensure the achieving of a satisfactory clinical response, and it may be that these patients require some alternative mode of treatment. At the moment, there is no method for determining the probability of successful low intensity, low total exposure PUVA therapy for any given patient. However, where this is successful it is so by 8 weeks of treatment (sixteen treatments; 25·6 J/cm^2) and such a course of treatment might be considered before transferring to the high intensity programme.

In our study there was no defined relationship between 2-h plasma levels of 8-MOP and response to PUVA therapy. However, special pharmacokinetic studies have shown abnormal plasma profiles for 8-MOP in four out of six non-responders and a poor response to PUVA may be due to such an abnormal pharmacokinetic behaviour (Stevenson *et al.*, 1980).

UV-B phototherapy, with natural sunlight or artificial sources, alone or in combination with coal tar or dithranol, has been used for many years in the treatment of psoriasis with varying degrees of success. Recent controlled studies have shown that UV-B alone is an effective agent in the resolution of psoriatic plaques and psoriasis generally (Fischer, 1976; Petrozzi *et al.*, 1978; Le Vine, White & Parrish, 1979; Le Vine & Parrish, 1980). Moreover, in a number of comparative studies results obtained with UV-B phototherapy were little different from those with PUVA therapy (Tronnier & Heidbuchel, 1976; Fischer, 1977; Pullmann, Wichmann & Steigleder, 1978; van Weelden, Young & van der Leun, 1980).

However, Boer *et al.* (1980) reported that UV-B phototherapy was successful in only thirty-three of forty-five patients, ten being dropped from the treatment schedule because of insufficient response. Similarly, Larkö & Swanbeck (1979) found that solarium unit treatment daily produced a satisfactory response in twenty of twenty-eight patients, and eleven of these twenty relapsed within 3 months despite twice weekly maintenance treatment.

The results which we obtained would also suggest that satisfactory improvement can be obtained with UV-B phototherapy if the exposure dose schedule is designed to take into account the 'tolerance' induced by each exposure. If the exposure dose is not increased at each treatment, the effect of the therapy is reduced and may be negated. With twice weekly exposure we were able to achieve a satisfactory response in twenty of twenty-five psoriasis patients who had previously been treated by conventional methods after a mean of 10·6 weeks. However, this satisfactory response was not maintained in all twenty patients during the continued treatment as it was for patients being treated with either low or high intensity PUVA. When the treatment was stopped, nine had relapsed to a varying extent. Of the sixteen patients classified as satisfactory when treatment was stopped, eight remained in remission for more than 8 weeks. It was possible to produce a significant improvement with UV-B in some patients who had relapsed after previous treatment with PUVA. However, the overall results were not so good in that only nine of the twenty-five patients were classified as having a satisfactory response on stopping treatment at a mean of 23·4 weeks. The maximum time of remission at the time of writing is 26 weeks, but only four patients have maintained this satisfactory state for more than 7 weeks after stopping treatment.

In summary, therefore, it would seem that the conventional high intensity photochemotherapy with 8-MOP and UV-A produces the most satisfactory clinical response. However, in a significant number of patients it is possible to produce that degree of satisfactory response with a low intensity PUVA regimen. Whether or not those patients who failed to respond to the low intensity irradiation would have done so if exposed initially to the high intensity regimen is uncertain. Similarly, UV-B phototherapy may be used successfully in a number of cases even though there appears to be a greater possibility of success with PUVA. Although some poor responders to PUVA may exhibit an abnormal pharmacokinetic handling of 8-MOP, there was no general relationship between the plasma levels obtained and the success of the treatment. Similarly, we were unable to detect the factors which determined the success or failure of low intensity PUVA or UV-B phototherapy. In time, it should be possible to define clinical criteria for the selection of patients for initial treatment with low intensity PUVA or UV-B phototherapy so that the higher total dosage irradiation procedure could be reserved for patients who require the high intensity PUVA regimen from the beginning of treatment. The problem of the relative safety of these alternative regimens remains unresolved.

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Effects of PUVA on delayed hypersensitivity in the guinea-pig

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SUMMARY

Guinea-pigs previously sensitized to dinitrochlorobenzene (DNCB) were topically treated with 8-methoxypsoralen solution and longwave ultraviolet light (PUVA). The animals showed moderate or no clinical and microscopic responses to an elicitation dose of DNCB given immediately after the period of treatment. Exposure to ultraviolet light or psoralen alone did not affect the allergic response. Ten days after PUVA treatment there was a normal eczematous reaction after application of DNCB.

Photochemotherapy using psoralen and longwave ultraviolet light (PUVA) has been established as an effective treatment of psoriasis (Parrish *et al.*, 1974). This method also seems to be useful for the treatment of other conditions, especially of diseases involving the immune system. Volden, Molin & Thomsen (1978) found that contact dermatitis to mechlorethamine in mycosis fungoides patients disappeared during PUVA therapy. PUVA has been shown to have a toxic effect on the immunocompetent cells in the epidermis (Morhenn, Benike & Engleman, 1980) and to inhibit cell mediated immune responses. Langerhans cells seem to play a central role in contact dermatitis by taking up and presenting antigen to T-lymphocytes. Aberer *et al.* (1980) showed that a UVA-dose of 100 J/cm^2 results in a dramatic reduction of Langerhans cells in human epidermis.

The many reports on the influence of photochemotherapy and UV-A on the immune system have prompted this investigation of the effects of topical PUVA and UV-A on contact dermatitis in the guinea-pig.

MATERIALS AND METHODS

Hartley strain albino female guinea-pigs weighing 350–400 g were used. Contact sensitivity to dinitrochlorobenzene (DNCB) was induced by a combination of intradermal and epidermal sensitization. This method gives a very high rate of sensitization (Magnusson & Kligman, 1969). An injection of 0·1 ml of 0·01% DNCB in propylene glycol was made intradermally. One week

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later closed-patch topical induction was performed. A 2 × 2 cm filter paper saturated with 0·15 ml 0·1% DNCB in 70% ethanol was applied to the clipped and shaved dorsal thorax. This was covered by an overlapping impermeable plastic tape, and held in place for 48 h by elastic adhesive bandage encircling the trunk. Challenge was performed ten days later by closed patch with 0·05 ml 0·1% DNCB in 70% ethanol. This was applied to the dorsal thorax in a Finn Chamber (Epitest Ltd, Finland) and fixed with porous adhesive tape and elastic bandage. The patch was removed after 24 h and the reactions were evaluated on the following day. The reactions were graded: 0 = negative; 1+ = slight erythema; 2+ = strong erythema; 3+ = intense erythema and swelling. Fifteen animals with 2+ or 3+ reactions were divided into three groups.

Group A

Seven animals were given topical PUVA treatment. An area of 6 × 6 cm on the right flank was clipped and shaved. One hour after application of 0·7 ml of 0·15% 8-methoxypsoralen solution, the area was exposed to 2·5 J/cm^{2} UV-A (Sylvania F 20 WIPUVA). The UV-A irradiance (320–390 nm) on the treated area was 6 mW/cm^{2}. This was repeated every other day for 16 days with a total UV-A dose of 20 J/cm^{2}.

Group B

Four animals were given the same treatment without 8-methoxypsoralen.

Group C

Four animals were given the same treatment as group A but without UV-A-exposure.

Immediately after the last treatment, all animals were exposed to an elicitation dose of DNCB by a Finn Chamber closed patch technique for 24 h, as described above. This was performed both on the right, treated flank and the left, untreated flank. The responses were evaluated 24 h after removal of the patches, and the test sites were also biopsied for histological evaluation. Ten days later the PUVA treated animals (group A) were re-challenged with DNCB following exactly the same procedure.

RESULTS

Five of seven animals in group A did not respond to an elicitation dose of DNCB applied on the PUVA-treated area, while two animals had a weak 1+ reaction. On the left, untreated side, they all responded with a strong 2+ or 3+ reaction (Table 1). Animals treated with UV-A or psoralen alone responded with a 2+ or 3+ reaction on both sides.

TABLE 1. Contact allergic reactions to DNCB immediately after treatment with PUVA

Group A		Group B		Group C	
Untreated side	PUVA side	Untreated side	UV-A side	Untreated side	Psoralen side
1. ++	–	1. ++	++	1. ++	+++
2. ++	–	2. +++	+++	2. ++	+++
3 +++	–	3. +	++	3. +++	++
4. +++	+	4. ++	+++	4. +++	+++
5. ++	–				
6. ++	+				
7. ++	–				

When re-challenged 10 days after cessation of PUVA treatment, six animals in group A gave strong (2+) reactions on the PUVA-treated side; the seventh gave a 1+ reaction (Table 2). Histological examination of the challenged sites of which no reaction occurred showed normal dermis and epidermis. In the two PUVA-treated animals with 1+ reaction there was moderate upper dermal lymphocyte infiltration. Biopsies of the challenge sites with stronger (2+, 3+) reactions showed spongiosis and lymphocyte infiltration in epidermis and vasodilatation and marked lymphocyte infiltration in the upper dermis.

TABLE 2. Contact allergic reactions to DNCB 10 days after PUVA treatment

Group A	
Untreated side	PUVA side
1. + + +	+ +
2. + +	+ +
3. + + +	+ +
4. + +	+ +
5. + + +	+ +
6. + +	+ +
7. + +	+

DISCUSSION

The present study demonstrates that PUVA therapy diminishes or abolishes an established cell-mediated reactivity to DNCB in the skin of the guinea-pig. This effect is temporary and can only be demonstrated in the treated area of the skin.

PUVA therapy of patients with allergic contact dermatitis improves the dermatitis, and patch tests demonstrate a diminution of the allergic reaction after therapy (Thorvaldsen & Volden, 1980). In psoriasis patients receiving PUVA therapy, there was found either abnormally low or undetectable delayed hypersensitivity reaction in the skin, as judged by the DNCB test (Strauss *et al.*, 1980). The results of the present guinea-pig study confirm the reports on human reactions. The guinea-pig provides a good model for further investigations on the effects of PUVA.

The mechanism of action of PUVA on cellular hypersensitivity is not completely understood. In the afferent phase of the immune response the antigen is presented to immunocompetent lymphocytes. There is now circumstantial evidence that the Langerhans cells are responsible for this function (Stingl *et al.*, 1980; Braathen, 1980). Langerhans cells disappear from the epidermis after exposure to UV-A irradiance of 100–160 J/cm^2 (Aberer *et al.*, 1980). It is likely that with the addition of psoralen this effect would occur at a much lower UV-A dose.

Concerning the efferent phase, the intensity of the skin reaction in contact sensitivity reflects the number of effector cells present if the concentration of hapten used for challenge is kept constant. Suppressor T-lymphocytes limit the number of effector cells (Polak, 1978) and regulate the immune response. Furthermore it has been demonstrated that PUVA induces specific suppressor lymphocytes (Roberts, Schmitt & Daynes, 1979).

All this indicates that PUVA inhibits both the afferent and efferent pathways in the cell mediated immune response. In the clinical response to PUVA therapy the inhibition of DNA synthesis in fibroblasts and keratinocytes is probably also of importance. However, the immunocompetent cells are far more sensitive to PUVA than fibroblasts or keratinocytes.

The actions of PUVA are dose-dependent (Kruger, Christophers & Schlaak, 1978), and this study has shown a limited, temporary effect. A PUVA dose of 20 J/cm^2 did not give any significant reduction of the immune response after 10 days. Whether this is due to a temporary suppression of the immunocompetent cells, or to the migration of new cells to the PUVA treated skin is not known.

ADDENDUM

Since the above paper was submitted for publication, similar findings have been reported from Boston (Morison *et al.*, 1981).

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Direct immunofluorescence on cytological smears in oral pemphigus

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SUMMARY

Direct immunofluorescence was performed on washed oral epithelial smears from thirteen patients with pemphigus vulgaris, thirteen patients with other oral diseases and from ten subjects with clinically healthy oral mucosa.

The intercellular deposition of IgG was observed on cytological smears from oral lesions in all patients with active pemphigus. In contrast, smears from pemphigus patients in remission, from patients with other oral diseases and from healthy controls, did not show any fluorescence. Therefore, direct immunofluorescence on cytological smears may be of value in the diagnosis of pemphigus.

Pemphigus vulgaris is an autoimmune bullous disease involving the skin and mucous membranes. In this condition, serum antibodies which react with an intercellular substance of stratified squamous epithelium can be detected by direct and indirect immunofluorescence.

Recently, intercellular deposition of IgG was demonstrated on cytological smears from oral pemphigus lesions (Decherd *et al.*, 1972). However, it was also reported that smears from other oral lesions (mainly aphthous ulcers) showed intercellular fluorescence similar to pemphigus (Skeete, 1977).

The aims of this investigation were to evaluate the specificity of immunoglobulin deposits on smears from pemphigus by direct immunofluorescence.

MATERIALS AND METHODS

Patients

Epithelial cells were obtained from the oral mucosa of thirteen patients with pemphigus vulgaris (seven males and six females) aged 48–82 years. Ten of these patients had active oral lesions and three were in remission. The diagnosis was confirmed by histology and by direct immunofluorescence on biopsy specimens. Furthermore, indirect immunofluorescence was performed in order to evaluate the serum intercellular (IC) antibody titre of these patients.

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Control epithelial smears were taken from seven patients with recurrent oral ulceration (aphthous ulcers), two patients with mucous membrane pemphigoid, three patients with erosive lichen planus, one patient with erythema multiforme and ten subjects with clinically healthy oral mucosa. The control patients were matched for age (46–84 years) and sex (thirteen males and ten females).

Collection and processing of epithelial cells

Before collecting the epithelial cells, the oral mucosa around the lesions was dried, the roof of the lesions, if present, was removed, and the base of the lesions was gently scraped with a wooden tongue depressor. The epithelial cells from healthy oral mucosa were scraped in the same way.

The cytological smears were prepared by two techniques:

(1) The scraped epithelial cells were spread directly on glass slides, air dried, rinsed for 1 min in PBS and used for direct immunofluorescence. Some slides were stained with haematoxylin and eosin to identify the acantholytic cells. The presence of acantholytic cells on unstained smears was also confirmed by phase contrast microscopy. For scanning electron microscopy, the scraped cells were spread directly on Agralon (ICI) polyester film.

(2) The cells were collected in 5 ml of tissue culture medium (TC-199, Wellcome Reagents) and washed twice by centrifugation (2000 r.p.m. for 10 min). Immediately after washing, the cells were counted and cytocentrifuged on to glass slides at a concentration of 4×10^4 per slide.

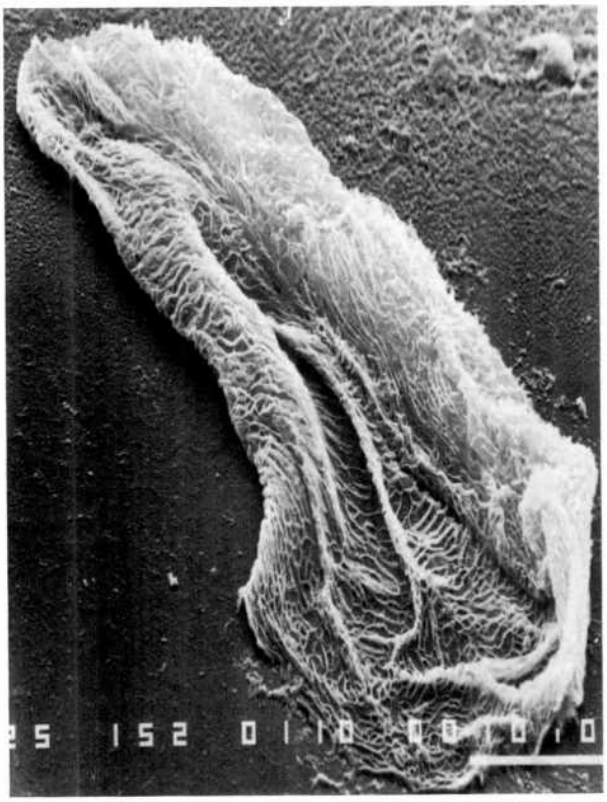

FIGURE 1. Topography of unwashed epithelial cell from healthy oral mucosa by SEM (× 1800).

For scanning electron microscopy, the cells were cytocentrifuged on Agralon polyester film by the same method.

Scanning electron microscopy

This was done in order to assess the effect of washing procedure on the topography of the epithelial cells. Cells were fixed in 3% glutaraldehyde in 0·085 M cacodylate buffer pH 7·4 for 90 min, rinsed three times with 5% sucrose in cacodylate buffer, dehydrated and immersed in acetone and critical point dried in a Polaron critical point drying apparatus (E.3000, Polaron Equipment Limited). Selected areas were mounted and coated with gold using argon in a Polaron SEM unit (E.5000, Polaron Equipment Limited). The cells were studied in a JEOL JSM-35 scanning electron microscope at 25 kV using a tilt angle of 60°.

The washing procedure did not affect the topography of oral epithelial cells (Figs 1 and 2). These cells showed surface details with no alteration of microridges.

Direct immunofluorescence

Immunofluorescent staining on unwashed and washed epithelial cell smears was carried out, according to the method of Coons & Kaplan (1950) with slight modifications. Briefly, the smears were washed for 30 min in phosphate buffered saline (PBS pH 7·4) and overlaid with FITC conjugated sheep antihuman immunoglobulins (Wellcome Reagents; anti IgG, F/P ratio 4·5 dilution 1:30; anti IgA F/P ratio 4·5 dilution 1:10; anti IgM F/P ratio 3·1 dilution 1:15). After incubation at 37°C for 30 min, smears were washed in PBS three times for 10 min each and mounted in buffered glycerol pH 8·6. All slides were examined immediately under a Leitz SM-Lux microscope equipped with a 50 W mercury lamp and the following filter system: heat filter KG-1, red suppressor filter LP-1, exciter filter K-490, barrier filter K515 and dichromatic mirror TK-510.

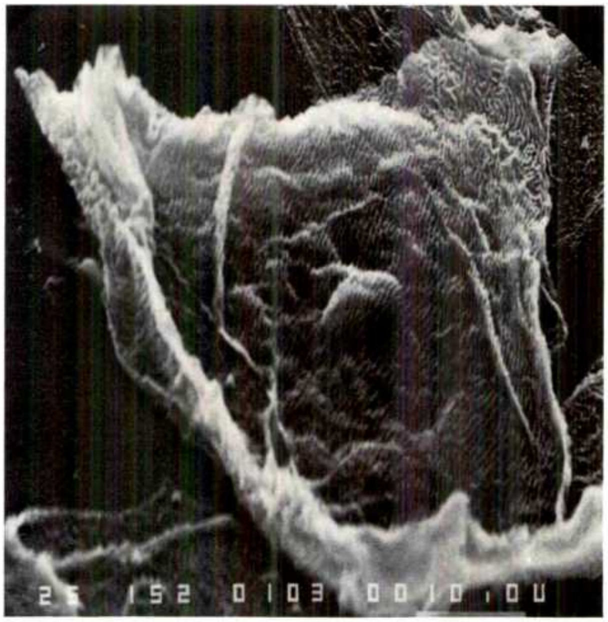

FIGURE 2. Topography of washed oral epithelial cell from healthy oral mucosa by SEM (× 1500).

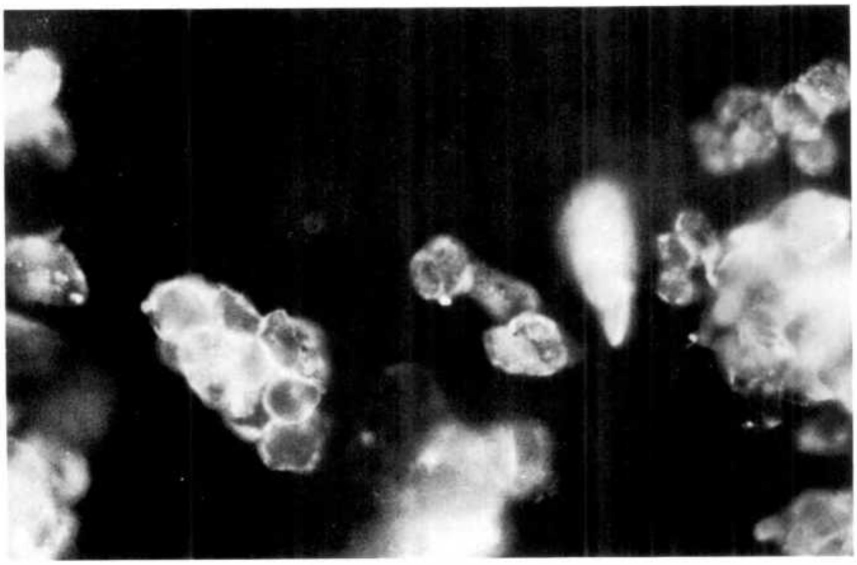

FIGURE 3. IgG deposition on unwashed epithelial cells from pemphigus lesion by immunofluorescence (× 700).

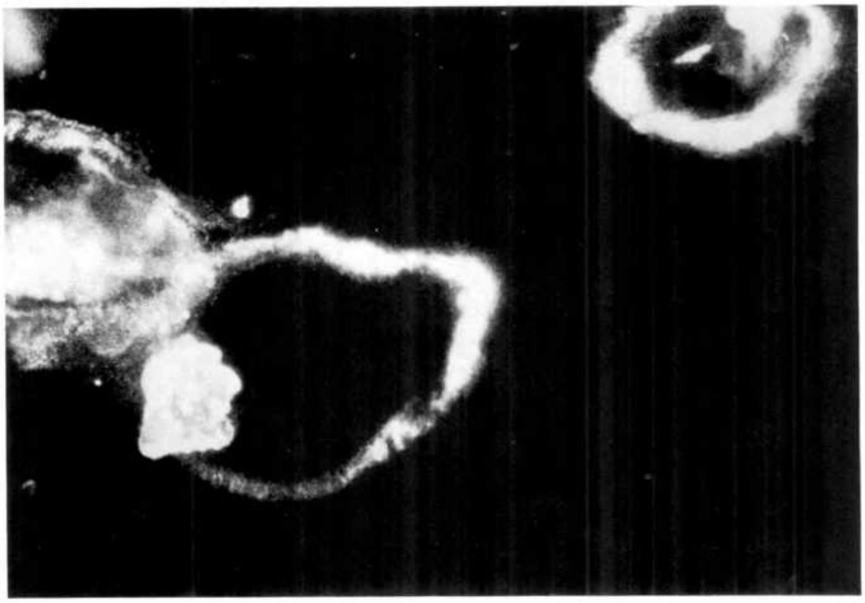

FIGURE 4. Immunofluorescence of acantholytic cells from pemphigus lesion with IgG after washing (× 1120).

Indirect immunofluorescence

Sera from thirteen patients with pemphigus were tested by indirect immunofluorescence (Coons & Kaplan, 1950) for the IC antibody titre using human skin as substrate.

RESULTS

Comparison of immunofluorescent patterns in unwashed and washed epithelial smears

Direct immunofluorescence in unwashed epithelial cells from pemphigus lesions showed a deposition of IgG around some individual acantholytic cells and an intercellular fluorescence on sheets of cells. However, many cells were in clumps and exhibited confluent fluorescence on the cell membranes (Fig. 3). This deposition of IgG on the cell membranes was also observed in the majority of smears from various oral lesions and in several smears from healthy oral mucosa. A few smears from patients with recurrent oral ulcers and erosive lichen planus showed intercellular fluorescence in some sheets of the cells.

In contrast, when washed and cytocentrifuged epithelial cell preparations were used, the cells were free of the cell membrane fluorescence and were evenly distributed on the slides with only few clumps present. Smears from pemphigus lesions showed the presence of individual acantholytic cells with a ring-shaped deposition of IgG around them (Fig. 4). Sheets of cells were also seen showing an intercellular fluorescent pattern (Fig. 5). Therefore, only washed and cytocentrifuged epithelial cells were used in the next experiment.

Deposition of immunoglobulins in oral epithelial smears

Ten patients with active oral pemphigus and three in remission were investigated (Table 1). All 'active' patients had serum IC antibody with titres ranging from 1:40 to 1:160. Intercellular antibody was not present in sera from two patients in remission, whilst one patient with recently

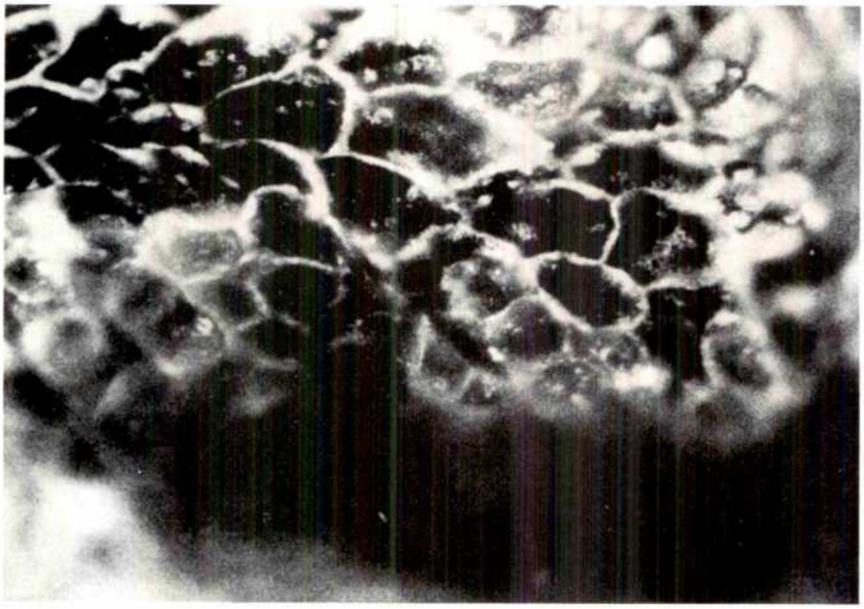

FIGURE 5. Intercellular IgG deposition of washed sheet of cells from pemphigus lesion (× 1120).

TABLE 1. Deposition of immunoglobulins in smears from oral pemphigus lesions

				Direct immunofluorescence		
Patient	Clinical status	IC*-antibody titre	Presence of acantholytic cells	IgG	IgA	IgM
1	Active	1:160	+	+	–	–
2	Active	1:160	+	+	–	–
3	Active	1:160	+	+	–	–
4	Active	1:160	+	+	–	–
5	Active	1:160	+	+	–	–
6	Active	1:160	+	+	+	–
7	Active	1:80	+	+	–	–
8	Active	1:80	+	+	–	–
9	Active	1:40	+	+	+	–
10	Active	1·40	+	+	–	–
11	Remission	1.10	–	–	–	–
12	Remission	Negative	–	–	–	–
13	Remission	Negative	–	–	–	–

*IC = intercellular.

healed lesions had a serum antibody titre of 1:10. Acantholytic cells were only seen in lesions from active pemphigus.

Direct immunofluorescence on epithelial cells from 'active' lesions showed IgG deposition in all ten patients. Smears from two patients (Nos 6 and 9) also showed intercellular IgA deposition. IgM positive fluorescence was not detected in any of the patients tested. Oral epithelial smears from three patients in remission did not show any IgG, IgA or IgM deposition. Finally, smears from thirteen patients with various oral diseases and from ten subjects with healthy oral mucosa showed no positive fluorescence for IgG, IgA or IgM (Table 2).

TABLE 2. Direct immunofluorescence on smears from various oral lesions and healthy oral mucosa

		Direct immunofluorescence		
Diagnosis	Number of subjects	IgG	IgA	IgM
Recurrent oral ulceration	7	–	–	–
Erosive lichen planus	3	–	–	–
Mucous membrane pemphigoid	2	–	–	–
Erythema multiforme	1	–	–	–
Healthy controls	10	–	–	–

DISCUSSION

The specificity of direct immunofluorescence for the detection of pemphigus antibodies in oral cytological smears has been a subject of controversy (Decherd *et al.*, 1972, Skeete, 1977).

The results show that the processing of scraped oral epithelial cells before direct immunofluorescence is of importance. Using unwashed cell preparations, an intercellular deposition of IgG was observed not only on smears from pemphigus lesions, but also in some sheets of cells from several patients with other oral diseases. Furthermore, the deposition of IgG on the cell membranes was seen in the majority of epithelial cell samples studied. Examination of unwashed smears by scanning electron microscopy showed that most of the cells scraped from oral lesions were coated with a film masking the cell surface details (results not presented). This seems likely to be due to non-specific attachment of serum proteins to the epithelial cell membranes. Coruh & Mason (1980) have demonstrated the intercellular and intracellular deposition of immunoglobulins and other serum proteins in various healthy and inflamed human epithelia. The most probable explanation is that they represent a leakage of serum proteins on and into epithelial cells (Coruh & Mason, 1980; Brandtzaeg, Surjan & Berdal, 1978). This has particular importance in the case of an inflamed tissue where the extravasation of serum proteins is increased. Another possible source of non-specific staining could be the mechanical trapping of immunoglobulins in clumps of cells (especially where the cellular borders overlap).

In contrast, washed and cytocentrifuged epithelial cells were free of the cell membrane positive fluorescence, and they were evenly distributed on the slides with only few clumps present. Furthermore, intercellular deposition of IgG was observed only in smears from pemphigus lesions. Interestingly, IgA localization was seen in intercellular substance areas in smears from two patients with pemphigus. Similar results were reported by other authors (Jordan *et al.*, 1974), but the role of IgA in the pathogenesis of pemphigus has not been elucidated.

Our results confirm the specificity of IgG deposits on smears from pemphigus lesions by direct immunofluorescence. This corroborates the initial report of Decherd *et al.* (1972) on the value of direct immunofluorescence on cell smears as an aid to the diagnosis of pemphigus vulgaris.

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Anti-gliadin antibodies and small intestinal mucosal damage in dermatitis herpetiformis

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SUMMARY

Sera from forty-six patients with dermatitis herpetiformis (DH) were examined for anti-gliadin antibodies (AGA) by the enzyme linked immunosorbent assay (ELISA) test and by a simple new immunofluorescent (IF) test. AGA were present in fifteen out of thirty-two patients taking a normal diet, but in none of the fourteen taking a gluten-free diet (GFD). The presence of circulating AGA was related to the severity of the enteropathy. AGA were present in all ten patients with a flat mucosa and in four of six with a convoluted mucosa, but in only one out of thirty patients with normal morphology of the small intestine. However, in those patients taking a normal diet and with a normal morphology of the intestine there was evidence of gluten sensitivity compared to those taking a GFD, as the intraepithelial lymphocyte count (IELC) was significantly raised in the peri-nuclear and supra-nuclear positions. The study shows that the presence of AGA in the serum is a good indication of the degree of gluten sensitivity as expressed by severe mucosal damage in patients with DH.

The association between dermatitis herpetiformis (DH) and gluten-sensitive enteropathy (GSE) is well recognized (Fry *et al.*, 1968; Shuster, Watson & Marks, 1968). The rash is gluten dependent (Fry *et al.*, 1973; Reunala *et al.*, 1977; Katz *et al.*, 1980), but the precise role of gluten in the pathogenesis of the skin lesions is unknown although an immunological mechanism has been postulated (Seah *et al.*, 1972).

AGA have been reported in a proportion of patients with DH (Kumar *et al.*, 1976; Eterman & Feltkamp, 1978; Menzel, Pehamberger & Holubar, 1978; Huff, Weston & Zirker, 1979), but these results have not been correlated with the degree of small intestinal abnormality in individual subjects. Comparison of the presence of AGA with small intestinal abnormality may give some indication as to the mechanism of production of these antibodies or their possible pathological role. In this study we investigated the incidence of AGA in a group of DH patients using a simple new IF test (Unsworth *et al.*, 1981a) and an enzyme-linked immunosorbent assay.

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PATIENTS

Serum samples were taken from each of forty-six unrelated Caucasian patients with DH and stored for up to one year at −20°C. There were twenty-nine men and seventeen women, aged from 15 to 78 years. A diagnosis of DH was based on the clinical and histological features, the response of the rash to dapsone and the presence of IgA in the uninvolved skin. In all forty-six patients the IgA deposits were papillary in distribution. Thirty-two were taking a normal gluten-containing diet and were treated by dapsone alone (50–400 mg/day), and fourteen had been controlled by a strict gluten-free diet (GFD) for at least 18 months. Serum samples from forty healthy hospital staff (twenty-five males and fifteen females aged between 20 and 62 years) were used as controls. Small intestinal biopsies were taken from the region of the duodenal-jejunal flexure with a Crosby capsule. The morphology was assessed using a dissecting microscope, and an inter-epithelial lymphocyte count (IELC) was performed as described by Fry *et al.* (1974).

MATERIALS AND METHODS

Preparation and crude gliadin

Crude gliadin was extracted from Flanders wheat flour (a variety widely grown in Great Britain) by direct extraction with 70% ethanol/water (v/v) for 2 h at room temperature. The insoluble material was spun down at 900 ***g*** and the supernatant dialysed with several changes against distilled water at 4°C overnight. Precipitated material was discarded, and the water soluble crude gliadin fraction was freeze dried. This crude gliadin was shown to contain the expected range of α, β, γ and ω gliadins, as well as minor wheat albumen band by polyacrylamide gel electrophoresis (Bushuk & Zillman, 1977).

ELISA method for detection of anti-gliadin antibody (AGA)

Gliadin antibodies in patients' sera were detected by a modification of the ELISA method described by Huff *et al.* (1979) using plastic micro-elisa plates (Dynatech Laboratories Ltd, U.K.), coated with 20 μg/ml of Flanders crude gliadin in 60% ethanol/water (v/v). Patients' sera were tested in triplicate at a dilution of 1/1000, and peroxidase labelled (polyvalent) sheep anti-human immunoglobulin (donated by Dr P.D.Weston, Wellcome Research Laboratories, Kent) was used at a dilution of 1/10,000. Optical densities were read at 492 nm in an ELISA reader (Dynatech Laboratories). Sera were tested on gliadin coated and uncoated (control) plates in parallel.

Anti-reticulin antibodies

Anti-reticulin antibodies (ARA) were detected as described by Seah *et al.* (1971). Sera were only considered positive for ARA if the R1 fluorescence pattern described by Rizzetto & Doniach (1973) was seen.

Immunofluorescence test for AGA.

Serum samples were screened for AGA on cryostat sections of composite rat tissue which had been preincubated for 30 min at room temperature with an aqueous solution of crude gliadin in distilled water (0·1 mg/ml). Unbound wheat protein was washed away in a bath of PBS for 15 min. Gliadin treated sections were then incubated for 30 min with patients' sera diluted in PBS, washed in PBS for 30 min and then incubated with class specific or polyvalent fluorescein labelled sheep anti-human immunoglobulin in the final step. AGA positive sera give a reticulin staining pattern strikingly similar to the RI fluorescent staining given by ARA on untreated rat tissue sections since gliadin binds to reticulin in tissue sections (Unsworth *et al.*, 1981b).

Fluorescein conjugated sheep anti-sera against human IgM, IgG, IgA and Ig (polyvalent), with fluorescein/protein (molar) ratios ranging from 3·1 to 4·0 were supplied by Wellcome Reagents and used at working dilutions determined by block titration (Unsworth *et al.*, 1981a). Slides were examined on a Reichert microscope equipped with a 100 W quartz halogen lamp and dark ground condenser. The primary filter was Balzar's FITC 3 and the secondary Wratten 12.

RESULTS

Incidence of AGA

AGA were detected in fifteen of the forty-six patients with the IF test and in twelve of these the ELISA test was positive (Table 1). No patient was positive on the ELISA test and negative by the IF test. There was a good correlation between the level of AGA by the ELISA and the titre of AGA by the IF except in three patients (5, 11 and 13). AGA were of IgG class in all fifteen patients and in addition, seven had IgA antibodies. Three of the fifteen patients also had ARA, but these were not found in any of the thirty-one patients who did not have AGA.

Relationship of AGA to intestinal morphology and IELC

Of the fifteen patients with AGA, fourteen had an abnormal macroscopic appearance of the mucosa. AGA were present in the ten patients who had flat biopsies and in four of the six

TABLE 1. Comparison of ELISA and immunofluorescent test for AGA in adults with DH

Patient	IF Polyvalent Ig	IF IgG	IF IgA	ELISA Polyvalent IG (%)	Small intestinal biopsy
1	> 1/1280	1/640	1/80	98	Flat
2	> 1/1280	1/320	1/40	100	Flat
3	1/640	1/320	1/80	48	Flat
4	1/320	1/320	−ve	37	Convoluted
5	1/640	1/320	−ve	4	Flat
6*	1/320	1/160	> 1/80	48	Flat
7*	1/320	1/160	> 1/80	41	Flat
8	1/160	1/160	−ve	30	Convoluted
9	1/80	1/80	−ve	20	Convoluted
10	1/80	1/80	1/40	16	Flat
11	1/80	1/40	1/10	2	Flat
12	1/80	1/40	−ve	15	Flat
13	1/40	1/40	−ve	2	Convoluted
14	1/40	1/20	−ve	40	Normal
15*	1/40	1/20	−ve	6	Flat

ELISA results are expressed as a percentage of a standard reference serum obtained from an untreated adult with coeliac disease and known to have high levels of AGA. The upper limit of normal for AGA in serum from normal controls by the ELISA test was 5% of the reference serum.

*ARA also detected (Patient No. 6, 1:80, No. 7, 1:40; No. 15, 1:40).

patients with convoluted biopsies (Table 2). Only one patient with a normal macroscopic appearance of the mucosa had AGA. In the fifteen patients in whom AGA were found, the mean IELC was 350 (Table 3), and in the patients taking a normal diet but in whom no AGA were detected the IELC was 325. Both of these figures are significantly raised compared to values for normal persons, but the mean count for those taking a GFD was 220 which is not significantly different from controls (Fry *et al.* 1974). The ratio of lymphocytes in the basal to the peri-nuclear position was 1:1 in those taking a GFD, but 1:2 in those taking a normal diet, irrespective of whether AGA were present (Table 3). The peri- and supra-nuclear counts were significantly raised in both the groups with and without AGA on a normal diet, but not in those on a GFD.

Relationship of AGA to diet

AGA were not found in the fourteen patients taking a strict GFD. They were present in fifteen of the thirty-two patients taking a normal diet.

TABLE 2. Relationship between the presence of circulating AGA in patients' serum as detected by IF and small intestinal morphology

Small intestinal biopsy	Number of patients	Number AGA positive
Flat	10	10
Convoluted	6	4
Normal	30	1

TABLE 3. Intra-epithelial lymphocyte count in relation to diet and presence of AGA (mean and S.E.)

	T	B	P	S
AGA +ve	350±35	117±11	229±28	5±1·4
AGA −ve (ND)	325±40	118±9	200±31	7±2·7
AGA −ve (GFD)	220±13	105±8	113±16	1±0·3
Control*	159±13	79±8	81±7	0·5±0·2

T = Total count.
B = Count of cells in the basal position.
P = Count of cells in the peri-nuclear position.
S = Count of cells in the supra-nuclear position.
ND = Normal diet.
GFD = Gluten-free diet.
* Control values (Fry *et al.*, 1974).

DISCUSSION

This study shows that AGA are present in approximately 50% of DH patients taking a normal diet. This is considerably higher than previous reports in which the incidence has ranged from 10 to 30% (Kumar *et al.*, 1976; Menzel *et al.*, 1978; Eterman & Feltkamp, 1977; Huff *et al.*, 1979). The higher incidence in the present study could be due to a number of factors. Firstly, there may be a qualitative difference in the wheat gliadin. Secondly, a difference in techniques: the IF test has not been used previously, but the ELISA test was used by Huff and his colleagues who detected AGA in only five of fourteen DH patients. Thirdly, if the presence of AGA does depend on a severely damaged intestinal mucosa, the incidence of a flat mucosa may have been lower in the other reports. However, apart from the report by Kumar *et al.* (1976) there was no attempt to correlate the incidence of AGA with the intestinal abnormality. In the study by Kumar the incidence of AGA was only about 10%, even in those patients with so-called subtotal villous atrophy.

In this study there was a good correlation between the intestinal morphology and the presence of AGA. All patients with a flat mucosa, and four of six with a convoluted mucosa, had AGA. This correlation between AGA and a severely abnormal mucosa could simply be due to leakage of gluten through the damaged mucosa, or alternatively they may in some way be involved aetiologically in producing the damage to the mucosa, i.e. as a gluten anti-gluten immune complex. In support of the hypothesis that they are due to simple diffusion through a damaged gut are the reports by Fordtran *et al.* (1967) and Wheeler, Menzies & Creamer (1978). In the latter study it was shown that in coeliac disease there is an increased permeability to large molecules compared to a normal small intestinal mucosa. Although the mucosa was morphologically normal in 50% of patients taking gluten in their diet, these patients do show evidence of gluten sensitivity in the intestinal mucosa as judged by a raised IELC and a 1:2 ratio of basal to peri-nuclear lymphocytes (Fry *et al.*, 1974). However, this mild degree of gluten sensitivity is not associated with AGA except in one of our patients.

There was good correlation between the IF test and the ELISA test with three exceptions (patients 5, 11 and 13). The IF test has the distinct advantage that it can readily be performed in laboratories already equipped to carry out other IF tests.

When AGA and ARA are present in the same serum they both contribute to the reticulin pattern of staining on gliadin treated sections. This was the case in three of our fifteen patients (Table 1). In two of these (patient 6, ARA titre of 1:80, ELISA binding 48%; patient 7, ARA titre of 1:40, ELISA binding 41%) enhanced reticulin pattern staining (1:320 in both) was seen when these sera were retested on gliadin-treated sections. Patient 15 (ELISA binding 6%) had an ARA titre of 1:40 which was not appreciably changed by retesting on a gliadin treated section. It is therefore likely in this case that the ARA is largely responsible for the positive staining given on the gliadin treated sections. However, the fact that ARA will interfere with the IF test for AGA is not in itself a serious disadvantage of the test, firstly because the incidence of ARA is only 15–20% in DH patients (Seah *et al.*, 1973), and secondly because the ARA correlates with intestinal abnormality, being more likely to be present in patients with macroscopic abnormality of the intestinal mucosa.

With the techniques described in this study, the presence of AGA is a good indicator of severe small intestinal mucosal abnormality, although their presence cannot be used as a screening test for DH as the incidence of macroscopic abnormality of the intestine in DH is only 50% (Fry *et al.*, 1974). However, AGA titre could be used to monitor the response to a GFD in those patients with severe mucosal abnormality, as Unsworth *et al.* (1981a) have shown that the titre gradually falls and the antibody finally disappears from the serum once the morphology of the intestine returns to normal in patients with GSE.

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Retinol binding protein in normal human skin and in cutaneous disorders

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SUMMARY

Circulating vitamin A is transported by a carrier termed retinol binding protein (RBP). Autoradiography and immunofluorescence studies showed that, in normal human skin, RBP is confined exclusively to the dermis. Following oral vitamin A loading, a large increase in dermal RBP but only a very small rise of serum RBP was found.

Serum RBP was slightly decreased in eczema patients and markedly decreased in patients with porphyria cutanea tarda. Values in other dermatoses, including psoriasis, were normal.

Circulating vitamin A (retinol) is transported by a specific carrier, called retinol binding protein (RBP). Under physiological conditions, RBP and retinol are bound in a 1:1 molecular ratio (Kanai, Raz & Goodman, 1968; Peterson, 1971a, b; Vahlquist, 1972). Small amounts of free, retinol-depleted RBP have been demonstrated, probably representing a transient form of the carrier undergoing catabolism (Peterson, 1971b, c; Rask, Vahlquist & Peterson, 1971). When bound to RBP, retinol is relatively stable, but free retinol is rapidly degraded by light, atmospheric oxygen or oxidizing agents (Kahan, 1971). The utilization of vitamin A seems to be dependent on RBP-specific receptors on target cells.

In the past years, vitamin A levels have been investigated in common dermatoses as well as in many experimental situations in which the skin is affected (Michaëlsson *et al.*, 1976; Mier & Van Den Hurk, 1974; Mier, Van Den Hurk & Van Rossum, 1975; Vahlquist, Michaëlsson & Juhlin, 1978). However, relatively little data are available regarding RBP in human skin. The present study was undertaken to investigate the distribution of RBP in healthy skin and to test the hypothesis that certain skin diseases might be associated with abnormalities in RBP metabolism.

MATERIALS AND METHODS

Autoradiography

Radioactive (^{125}I) labelled RBP was prepared according to Heller (1975) from normal human

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serum (specific radioactivity $5 \cdot 1 \times 10^5$ c.p.m./pmol) and from serum of patients suffering from retinitis pigmentosa (specific radioactivity $5 \cdot 9 \times 10^5$ c.p.m./pmol). After informed consent, 0·1 ml of each (^{125}I)-RBP was injected intradermally in two areas of the dorsal skin of two male, healthy control subjects, 25 and 46 years old. Under local deep anaesthesia, 4 mm punch biopsies were obtained from the injected areas, 10 and 60 min after injection. The biopsy specimens were fixed in Bouin fluid, embedded in paraffin and processed for autoradiography. The slides were developed after 25 days of exposure at 4°C.

Immunofluorescence

Direct and indirect immunofluorescence (IF) studies were performed on 4–5 μm frozen sections of normal skin (dorsum) of four normal male subjects, 27–38 years old. Two of the four subjects were biopsied after oral administration of high doses of vitamin A by mouth (600,000 iu daily for 10 days). For direct IF we used a rabbit antihuman RBP serum, conjugated with fluorescein isothiocyanate, diluted 1:10, 1:30 and 1:50. Indirect IF was performed by incubating the frozen sections with rabbit antihuman RBP serum (Behring) for 30 min at room temperature. After PBS washing, a swine antirabbit serum, conjugated with FITC (Dakopatt, Denmark) was added for 30 min. After PBS washing, the slides were mounted with buffered glycerine. Control sections were prepared according to the blocking method, by replacing the labelled antiserum with an unlabelled one. Records were obtained on Ektachrome 23 DIN colour film, using a Leitz Orthoplan fluorescence microscope with incident light, equipped with a Philips XBO 75 W lamp.

Serum RBP and vitamin A measurements

The levels of RBP and vitamin A were determined in sera from ten control subjects before and after vitamin A loading (300,000 iu daily for 5 days). These subjects (six males and four females, age range 18–60 years) had been hospitalized for minimal localized dermatological disorders such as common warts, nevi and small basal cell carcinomas. Sera were stored at −25°C in darkness prior to assay.

Serum RBP was also measured in eighty-five patients with various skin diseases (sixty males and twenty-five females). This group included diffuse, oozing eczema (twenty-one cases), psoriasis (twenty cases), porphyria cutanea tarda (twenty-one cases) and twenty-three 'miscellaneous' cases (pemphigus vulgaris, pemphigoid, varicose ulcer, furunculoses, mycoses and urticaria).

Serum RBP was estimated by the radial immunodiffusion technique, according to Mancini, Carbonara & Heremans (1965). Agar plates containing antihuman-RBP serum (M Partigen) were used, obtained from Behring Werke. All the sera were tested in duplicate, in comparison with three serial dilutions of a commercially available standard serum supplied by Behring Werke, containing 5·8 mg of RBP per 100 ml (Batch 1004 T). The immunoplates were read after 48 h, unstained, at room temperature, in a standard 'Transydine' calibrating viewer. A calibrated microsyringe (SMI micropettor 20 μl) was used to fill the wells of the immunoplates; the use of glass micropipettes led to erroneous results.

Serum vitamin A content was determined by a spectrofluorimetric method (Kahan, 1971) in a Perkin Elmer fluorescence spectrophotometer, model MPF-44A. All the control sera were assayed in duplicate in comparison with a standard vitamin A solution of crystalline all-trans-retinyl-acetate (Sigma), in cyclohexane.

RESULTS

Following intradermal injection of (^{125}I)-RBP, the silver granules appeared uniformly dense in the dermis. Occasional, scattered granules were detected on the epithelial structures; these were of about the same density as the normal background (Fig. 1). Due to a higher specific radioactivity, the number of the granules was greater in sections of skin injected with retinitis pigmentosa serum-RBP than in sections of skin injected with normal serum-RBP. No differences could be detected in the autoradiographic slides of skin biopsied 10 or 60 min after injection.

The dermis of subjects receiving vitamin A by mouth for 10 consecutive days was intensely fluorescent after direct and indirect IF procedures. No IF staining was obtained in the dermis of subjects not receiving vitamin A, nor was it detected in any epithelial structures (Fig. 2).

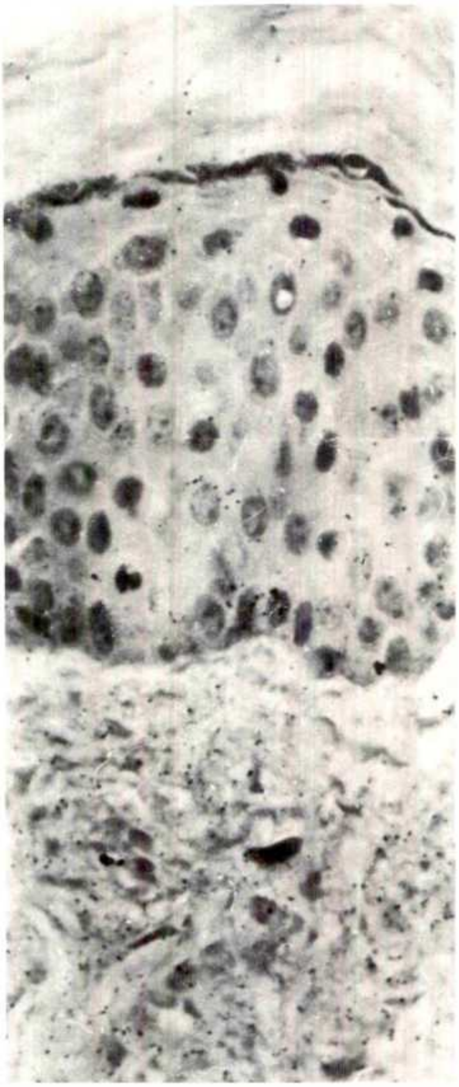

FIGURE 1. Autoradiography of normal human skin injected with (^{125}I)-RBP.

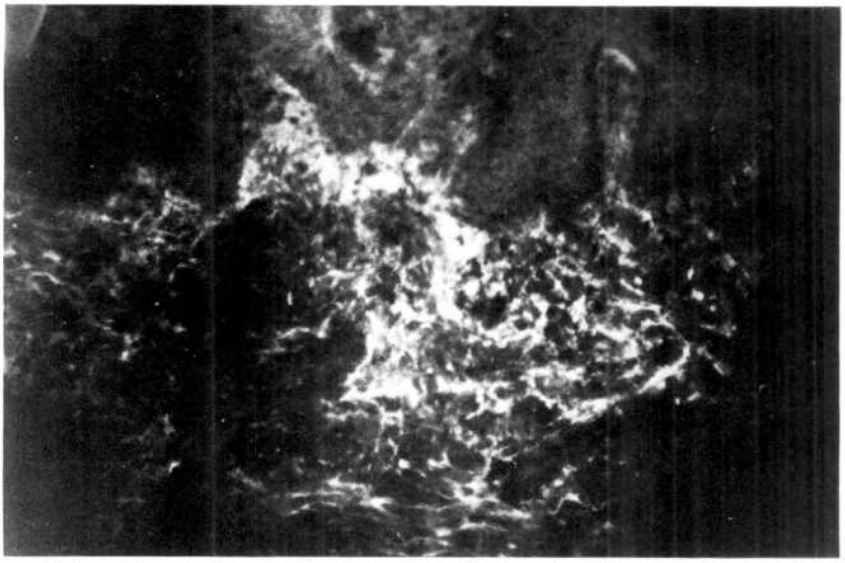

FIGURE 2. Indirect immunofluorescence of the dermis in control subject after vitamin A loading by mouth.

TABLE 1. Serum vitamin A and RBP levels in healthy controls, before and after vitamin A loading by mouth

	Basal	After vitamin A loading	Percent change	P
Vitamin A μg%	40·4 ± 7·1*	59 ± 10*	+46	<0·001
RBP mg%	6·4 ± 1*	7·5 ± 1·2*	+17	<0·1

* ± Standard deviation.

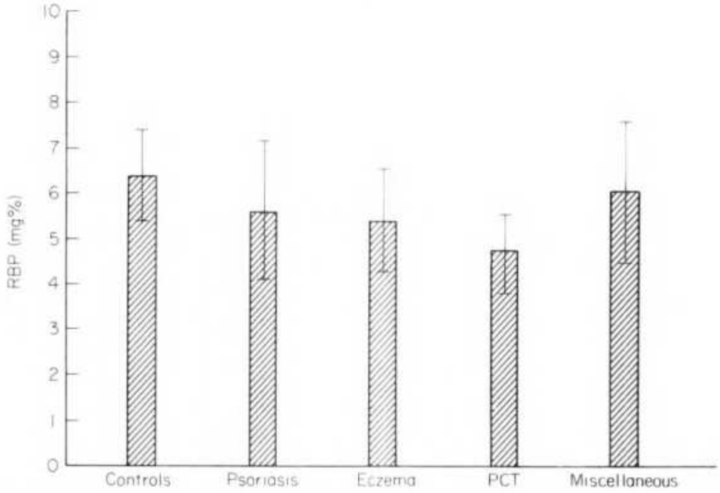

FIGURE 3. Mean serum RBP values in healthy controls and in various diseases.

It is seen from Table 1 that the serum vitamin A levels in the ten control subjects rose by 46% following loading. In contrast, the RBP levels increased by only 17%, a change of borderline significance ($P < 0{\cdot}1$). In comparison to the control subjects, RBP appeared not significantly decreased in psoriatic patients ($P > 0{\cdot}1$) or in miscellaneous cases ($P > 0{\cdot}6$). RBP was significantly decreased in eczema patients ($P < 0{\cdot}05$) and very significantly decreased in porphyria cutanea tarda patients ($P < 0{\cdot}001$) (Fig. 3).

DISCUSSION

Both our autoradiographic and immunofluorescent experiments indicate that RBP does not penetrate the epidermal layers of human skin. A similar situation has been reported in the bovine eye, where RBP receptors seem to be confined to the basal (choroidal) surface of the epithelial cells, and the labelled RBP molecule does not reach the cytoplasm (Heller, 1975; Maraini & Gozzoli, 1976; Bok & Heller, 1976). Presumably in human skin, as in the bovine eye, RBP attains the basal surface of the basal cell and thereafter its functional role of vitamin A carrier is accomplished. This hypothesis, however, requires further investigations: it is possible that the absence of labelled RBP in epidermal structures is due to an intrinsic lability of the linkage between the labelled RBP and membrane receptors or to the reagents used during the histological procedures.

Immunofluorescence studies on RBP have been extensively performed by Glover, Jay & White (1974) in liver and kidney and by Forsum *et al.* (1977) in human skin. In the liver, a specific fluorescence tightly bound to cytoplasmic organelles has been demonstrated. In the kidney, RBP appeared widely distributed in the intercellular spaces, as well as within the glomerular tufts. In human skin, in contrast to our own findings, the presence of specific RBP receptors was observed only on granular and horny cells of the epidermis.

Our measurements of serum RBP levels before and after 'loading' with vitamin A confirm the report of Vahlquist, Johnsson & Nygren (1979), and indicate only a very small rise in circulating RBP despite the remarkable increase in dermal fluorescence. This is compatible with the fact that the liver is the site of synthesis of RBP and contains a large store of the apo-protein. Following vitamin A uptake, the carrier is mobilized as olo-RBP (Peterson *et al.*, 1973; Smith *et al.*, 1973), the latter being rapidly taken out of circulation and deposited in 'target tissues' such as the dermis. It is of interest that serum RBP levels do show an appreciable increase following treatment of patients with hypovitaminosis A (Vahlquist *et al.*, 1978). Conversely, toxic doses of vitamin A have been reported to cause a reduction of serum RBP in rats (Mallia, Smith & Goodman, 1975).

We have demonstrated that serum RBP levels are significantly decreased in porphyria cutanea tarda. This may be caused either by decreased biosynthesis in the liver (Skrede *et al.*, 1975) or by an impaired absorption of vitamin A from the gut and reduced release of RBP from the liver (Prellwitz *et al.*, 1974). Studies on protein-calorie malnutrition (kwashiorkor), have shown that low values of RBP in this pathological condition seem to be the result of reduced liver biosynthesis (Ingenbleek *et al.*, 1975).

The occurrence of somewhat low levels of RBP in the serum of eczema patients could be correlated with the hypoalbuminaemia and hypoproteinaemia that usually characterize these patients, especially when diffusely oozing. The normal values observed in other dermatoses, including psoriasis, are consistent with the view that skin disease *per se* is not associated with a disturbance in the normal homeostasis of RBP.

ACKNOWLEDGMENTS

The authors are greatly indebted to Professor Maraini for supplying ^{125}I-RBP and ITCF-labelled RBP, as well as for criticism and advice. Thanks are due also to Professor Lars Rask for help with interpretation of the autoradiographic slides. The technical assistance of Miss Albertina Ollari was greatly appreciated.

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Host-parasite relationships in hyperkeratotic (Norwegian) scabies: pathological and immunological findings

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SUMMARY

The dynamics of intraepidermal penetration of the scabies mite and the host's responses to the invaders have been studied in hyperkeratotic scabies.

Contrary to the generally accepted view, the mites progressed not only through the stratum corneum but also into the underlying epidermis and even occasionally to the dermo-epidermal interface. Morphological evidence of reactive processes included parakeratosis surrounding the burrows, psoriasiform hyperplasia with increased basal cell labelling indices and exocytosis of mononuclear cells. ^{3}H-Thymidine labelling indices of immunocompetent cells of the dermal infiltrate reached levels found in allergic patch test reactions and were in both cases higher than in non-immune-specific inflammatory processes. The labelling index of peripheral blood lymphocytes showed an increased ratio of B cells to T cells as compared to controls or patients with allergic contact dermatitis. Serum C1q fixation was higher than in untreated patients with common scabies.

These results support our previous findings of immunological activation in hosts infested by the scabies mite.

The conditions which predispose to the development of hyperkeratotic scabies have been reviewed by Chouvet *et al.* (1979). In this paper we present the results of a study of the host-parasite relationship in this condition.

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CASES

We studied three patients:

(1) An 89-year-old woman was seen with a 10-month history of a generalized erythematous and pruritic eruption simulating erythroderma. Grey scales were present on the lateral aspects of the fingers and extended on to the palmar and dorsal aspects of the hands. Cracks and deep fissures with coagulated exudate were also seen (Fig. 1). The nails appeared unaffected. The entire surface of the skin including the face was erythematous, oedematous and covered with fine dusty hyperkeratosis. There were no obvious excoriations but some exudative areas were present. General examination of this senile psychotic patient disclosed no pathological findings except enlargement of inguinal lymph nodes. Before topical treatment was started the white blood cell count was 11,900 cells/ml (74% neutrophils, 15% eosinophils, 9% lymphocytes, 2% monocytes). Normal values were obtained with the following blood tests: sugar, electro- and immuno-electrophoresis of serum proteins, standard tests for syphilis, antistreptolysin O, vitamin A, immunoglobulins (IgG, IgM, IgA, IgE), and erythrocyte sedimentation rate.

Topical application of 10% salicylic acid ointment and of 10% benzyl benzoate emulsion was then started. Fifteen days later, the patient developed transient proteinuria (1 g/l) but renal function tests remained normal.

(2) An otherwise healthy 47-year-old man was seen for an 'itchy dermatitis'. He was treated for several months with topical steroids. Hyperkeratosis was present on the lateral aspects of both hands and feet in a pattern similar to that of patient 1. Dusty scaling was also present on the scalp and face. Nails were not involved. White blood cell count was 11,200 cells/ml (60% neutrophils, 20% eosinophils, 2% basophils, 16% lymphocytes, 2% monocytes). Erythrocyte

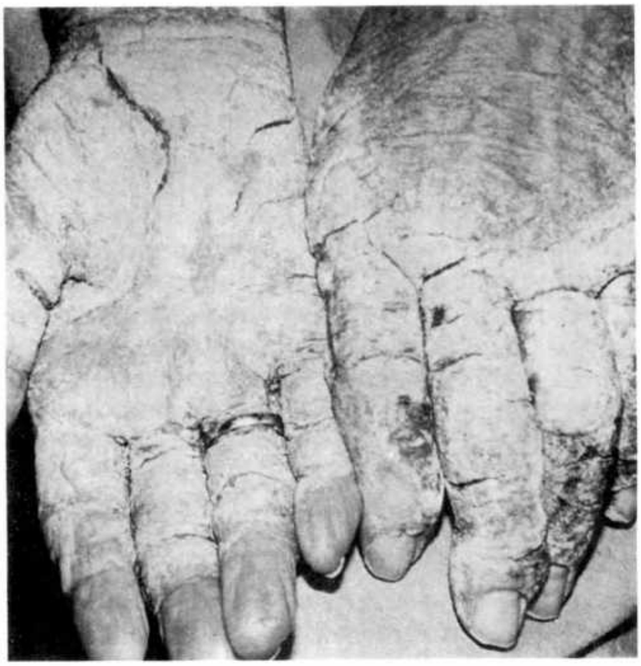

FIGURE 1. Hands of patient 1. Greyish brittle hyperkeratosis extends from interdigital spaces to dorsal and palmar aspects of the hands.

sedimentation rate was raised (30 mm/h). Serum protein profiles, standard tests for syphilis, immunoglobulins IgG, IgM, IgA and renal function tests were within normal levels. Once the skin investigation had been completed, successful topical treatment was applied as in patient 1.

(3) No clinical details were available for this patient. A skin specimen was sent to our laboratory and a histological diagnosis of hyperkeratotic scabies was made.

METHODS

Skin biopsies

Four millimetre punch biopsies were taken after local injection of lignocaine without adrenaline. Untreated sites were chosen on the lateral aspects of the thumb where the skin appeared hyperkeratotic and erythematous.

Histological procedures

After fixation in Bouin's fluid, 200 (or more) serial sections (6 μm thick) were performed on each specimen. Every other slide was stained by haemalum-eosin-saffron or by periodic acid-Schiff.

Immunofluorescence

Biopsies of patients 1 and 2 were snap frozen in liquid nitrogen and stored at -70°C until processing for direct (DIF) and indirect (IIF) immunofluorescence. The specimens were cut at 5 μm with a cryostat (-20°C). The sections were used unfixed. FITC labelled goat anti-human IgG, IgA, IgM, IgE, C3 and fibrinogen were purchased from Hyland, anti-C1q from Behring and anti-C4 from Meloy. Each antiserum was used diluted at 1/20 in phosphate buffered saline (PBS).

The substrates used for IIF were the lesional skin of HS patients and rabbit lips. The sera of the patients were diluted at 1/2 with PBS and incubation on the substrates was allowed for 30 min at room temperature. These slides were rinsed in PBS (10 min). Thereafter they were processed as for DIF with the aforementioned antisera. After 30 min incubation at room temperature, slides were washed in PBS and mounted in PBS with 10% glycerol.

^{3}H-Thymidine labelling indices in the skin

One 4 mm biopsy was cut with a razor blade into four 1 mm thick slices. The slices were incubated for 2 h at 37°C in a culture medium containing 2 μCi/ml of tritiated thymidine (^{3}HT, specific activity 10 Ci/mmole; 1 mCi/ml; Centre d'Énergie Nucléaire, Mol, Belgium) according to the method described by Lachapelle & Gillman (1969). After incubation the specimens were processed for radioautography. Labelling indices (LI) are the relative percentage of labelled cells (more than ten silver grains per nucleus) within a sample of 1200 basal cells of the epidermis (basal labelling index, BLI), and 2000 mononuclears of the perivascular cell infiltrate in the upper dermis (dermal labelling index, DLI). These LI were compared to those obtained in normal skin of the thumb (Lachapelle, 1970) and those obtained after application of irritant substances on the skin or in positive delayed type hypersensitivity reactions seen 48 h after application of haptens on the skin of sensitized patients (Lachapelle, 1972; Van Neste & Lachapelle, 1980).

^{3}H Thymidine labelling indices of lymphocyte populations in the peripheral blood

Twenty millilitres of blood were collected on heparin in patient 1 and 2 prior to any treatment, 40 μCi of ^{3}HT were added to each blood sample and incubation was allowed for 1 h at room temperature. After separation on Ficoll gradients peripheral blood lymphocytes were processed for E rosette formation (ER test). The slides obtained by cytocentrifugation of the cell suspensions were processed for radioautography. Cells surrounded by three or more sheep red blood cells were termed rosette-forming cells (RFC). The labelling index is the relative percentage of labelled cells (more than ten silver grains per cell nucleus) in a sample of 2000 lymphocytes. Monocytes were excluded by morphology. The ratio (labelled RFC/labelled NRFC) was determined in each patient. The values were compared to the levels obtained in ten healthy subjects (De Bruyere, Lachapelle & Anguita, 1977), in five patients with widespread allergic contact dermatitis (Lachapelle *et al.*, 1980) and in ten patients with atopic dermatitis (Van Neste & Lachapelle, 1981).

Scanning electron microscopy (SEM)

Crusts were taken from the lateral aspect of the hands and fixed in Bouin's fluid. Specimens were air-dried, mounted on SEM stubs, gold coated and examined under 30 kV with a Philips SEM 501.

Serum C1q binding activity (C1q BA)

The presence of circulating antigen-antibody complexes able to bind C1q was determined by the C1q BA test as described by Zubler *et al.* (1976). Results obtained in controls and in common scabies before and after treatment have been published previously (Van Neste & Salmon, 1978) and will be used for comparison to those obtained in HS.

RESULTS

Histology

At first glance, massive hyperkeratosis, psoriasiform hyperplasia and spongiotic alterations are obvious epidermal changes (Fig. 2). On closer examination, the upper parts of the epidermis appear hollowed by numerous burrows. The junction of the cornified layers with the underlying epidermis is irregularly depressed and cellular structures are altered. These changes can be ascribed to the presence of burrowing mites. Indeed cytolysis is clearly seen within the epidermis some 30 to 80 μm ahead of the body of the mite. This is observed on longitudinally sectioned burrows (Fig. 3) or on transverse sections (Fig. 4). The very first stages of the burrow formation are characterized by cellular alterations affecting at first only some epidermal cells, but soon involving an area sufficient to allow the progression of the mite's body. The bottom of the burrow, either within the stratum corneum or within the deeper parts of the epidermis, is made of an amorphous gel resulting from a lytic effect induced at a distance by the scabies mite.

When the necrotic process occurs within the normal horny layer, the walls of the burrow are and remain orthokeratotic (Fig. 5). As soon as the mite ploughs into the underlying epidermis, disturbed keratinization of non-lethally affected cells surrounding the necrotic area results in parakeratosis. This is observed at first only on the floor of the burrow, the roof remaining as is, i.e. orthokeratotic, but with increasing depth of grooving, parakeratotic cells constitute the floor and lateral walls of the burrow. Finally when the ploughing mite is wholly within the malpighian layers, the burrow is surrounded exclusively by parakeratotic cells. Subepidermal blisters were occasionally present at the tips of dermal papillae, near to the top end of the burrow (Figs 4 and 6). Besides cytolytic effects presumably induced by the mite, the epidermis is hyperplastic with deeply interdigitating epidermal rete ridges. Exocytosis of mononuclear cells and other leucocytes is similar to that encountered in lesions of psoriasis. This occurs very often close to the secretion products of the parasite. The superficial dermis is

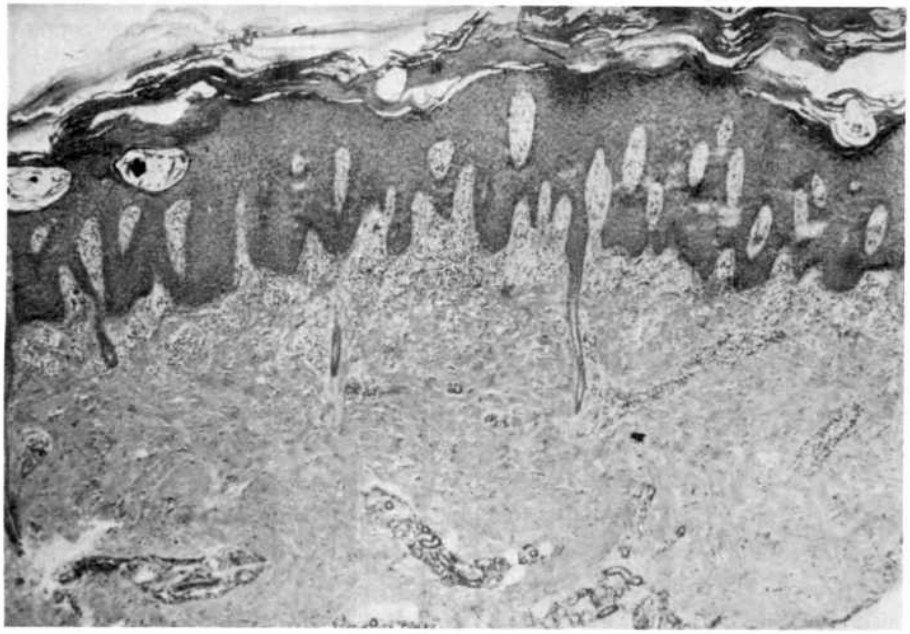

FIGURE 2. Scanning power view of skin lesions in hyperkeratotic scabies (hematoxylin, eosin and saffron stain). Hyperkeratosis, psoriasiform hyperplasia of the epidermis and superficial mixed cell infiltrate characterize the skin lesions. Junction between horny layer and granular or Malpighian layers is irregularly depressed by burrowing mites.

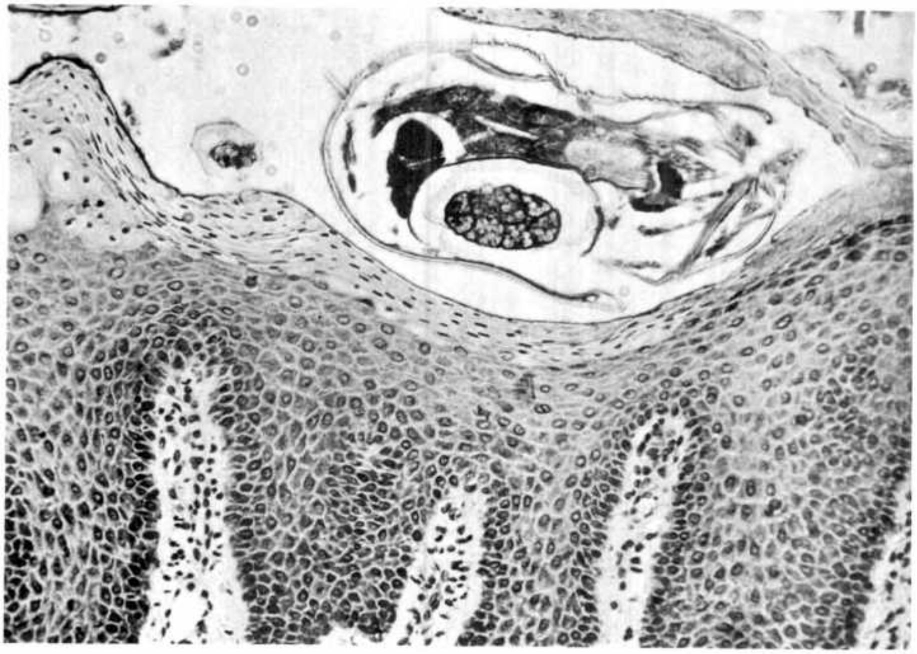

FIGURE 3. Histogenesis of the burrow: longitudinal section through a burrow. The granular layer, present on the right of the figure, is destroyed at some distance by the mite which progresses from the left to the right. Cells in the floor of the burrow, undergo parakeratotic cornification. Parakeratosis is already visible under the body of the mite and is more pronounced under the egg (left). Eggs are laid through a transverse cleft in the ventral surface. The linings of this cleft called the tocostome, usually tightly joined, appear widely opened during egg laying.

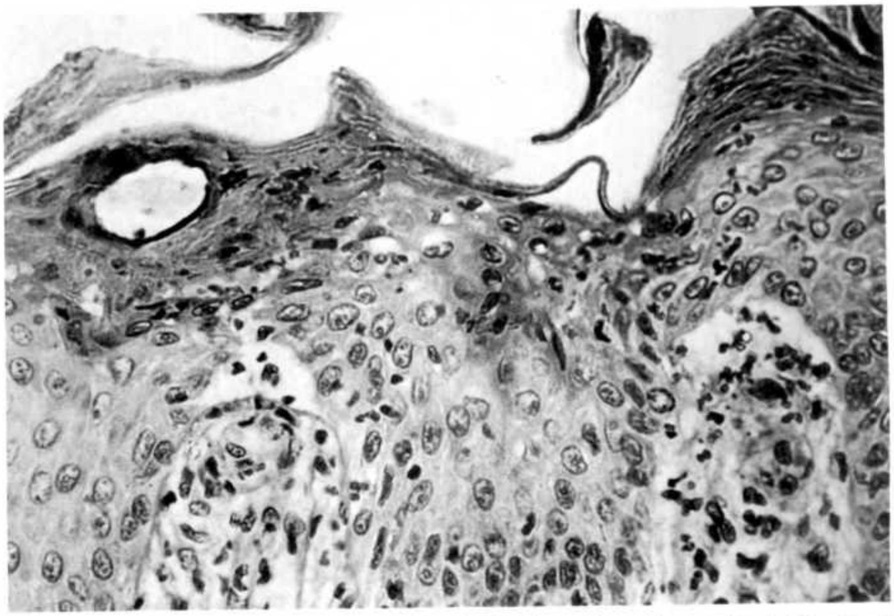

FIGURE 4. Histogenesis of the burrow: the intra-Malpighian phase. Some microns ahead of the ploughing mite, epidermal cell alterations and cellular necrosis are observed (centre). On the left of the figure, another mite tunnels on and appears surrounded by this necrotic material. Disturbed keratinization of the surrounding epidermal cells will result in parakeratosis. Blisters are observed at the tips of the dermal papilla where a large number of mononuclear cells migrate from the perivascular areas into the altered epidermis.

oedematous, and infiltrated by a large number of lympho-histiocytic cells. The density of the dermal infiltrate seems to be related to the presence of mites and to the depth of ploughing. The dermo-epidermal junction is thickened and specific deposition of C3 in a band-like pattern was observed in patient 1. The life cycle of the parasite can easily be studied in the epidermis of HS. In one section we had the opportunity to observe how eggs are laid down by the female through the tocostome (transversal ventral cleft) which is usually closed (Fig. 3). The eggs, embedded in an amorphous material lie in tiers on the floor of the burrow. When larval maturation has been completed, the egg shells are cut out from inside (Fig. 7) and mature larvae leave them. Later on, the empty shells will be shed with the surrounding corneocytes. A detailed study on the life cycle of the parasite is now in progress.

Autoradiography after ^{3}HT labelling (Table 1)

Epidermis. By comparison with controls, there was an increase of the ^{3}HT labelling index of the basal cells of the epidermis in our two patients with HS.

Dermis. The dermal labelling indices of patient 1 (and to a lesser extent of patient 2) were comparable to those observed in positive allergic patch test reactions; they were higher than the labelling of the infiltrate obtained after application of irritant substances on the skin (Lachapelle, 1972; Van Neste & Lachapelle, 1980).

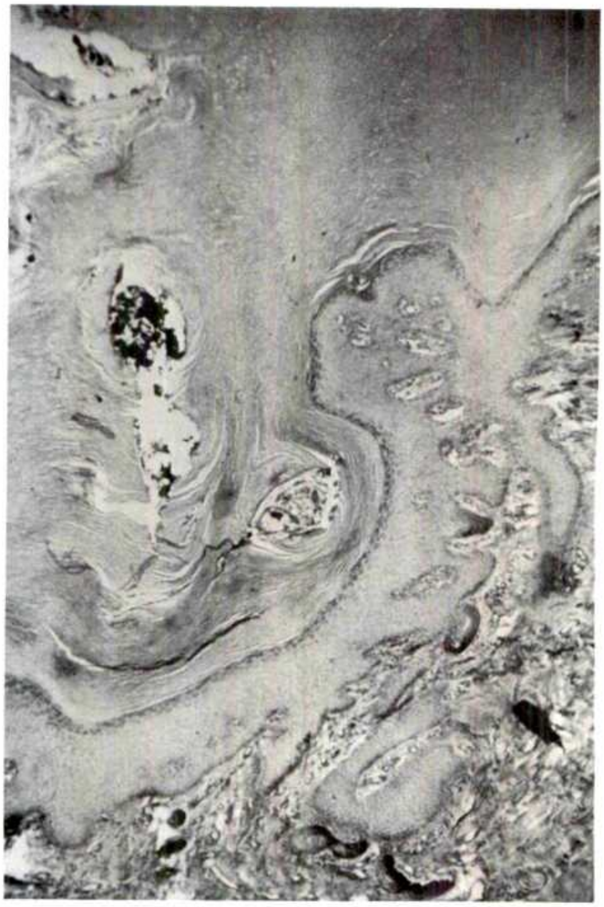

FIGURE 5. Histogenesis of the burrow: the intracorneal phase of the process. To reach the living parts of the epidermis, the mite has to perforate the horny layers. This very first step of the invasive process is easily observed on the palmar aspect of the hands. Two mites are seen burrowing in the thick orthokeratotic layers. As no metabolic activity exists at this level, and, as a rule, could not be altered by the lytic process induced by the mite, the walls of the burrow remain orthokeratotic.

Peripheral blood lymphocytes

An increased blastogenesis was observed in the peripheral blood lymphocytes of our two patients with HS; RFC (T cells) and NRFC (B cells) were involved in this reaction as suggested by the ratios (LI-RFC/LI-NRFC) which were 1·4 and 0·7 in patients 1 and 2 respectively. Comparatively, this ratio in widespread allergic contact dermatitis was 3·09 (Lachapelle *et al.*, 1980) whereas in atopic dermatitis it was 0·89 (Van Neste & Lachapelle, 1981).

DISCUSSION

The mode of intra-epidermal progression of the scabies mite, illustrated here in three cases of hyperkeratotic scabies, is identical to that published by Heilesen (1946), who clearly showed the top end of the parasite sticking in the living or 'wet parts' of the epidermis. This aspect is

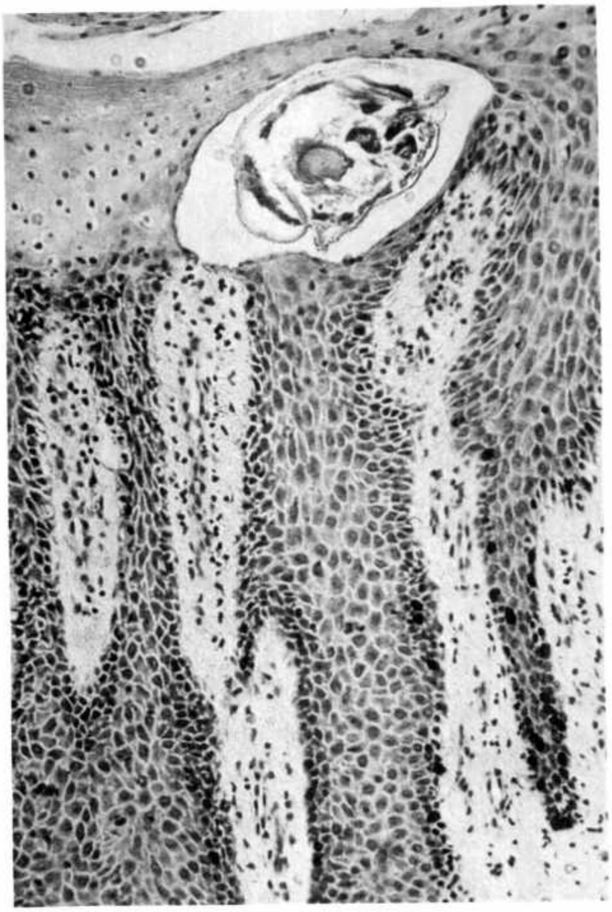

FIGURE 6. Histogenesis of the burrow: mite at the dermo-epidermal interface. Occasionally mites destroy basal cells and reach the dermo-epidermal interface. Collagen fibres seem unaltered by the invasive process and hinder progression of the parasite. In any case, the mite moves on and turns to more favourable areas (see forelegs which indicate progression to the right side of the figure).

thought to be uncommon in ordinary scabies (Hejazi & Mehregan, 1975; Fernandez, Torres & Ackerman, 1977) and has been overlooked in most papers dealing with cases of hyperkeratotic scabies although it could be clearly identified on most of the histological pictures reproduced therein (Burks, Jung & George, 1956; Maguire & Kligman, 1960; Schiff & Ronchese, 1964; Schirren, 1970; Haydon & Caplan, 1971; Espy & Jolly, 1976; Ackerman, 1977; Fernandez *et al.*, 1977).

Intracorneal and intramalpighian localization of the body of the mite is only the later stage of the process, the very first event of which is cellular destruction occuring at some distance in front of the progressing mite. Cellular debris, resulting from this lytic process probably provides the unique nutrient medium for the scabies mite. Ingestion of this necrotic material allows the mite to progress forwards leaving an empty space, which constitutes the usual burrow in ordinary scabies, where eggs and faecal material are to be found. When this lytic process occurs in the already formed horny layer, the walls of the burrow remain orthokeratotic. Conversely when the

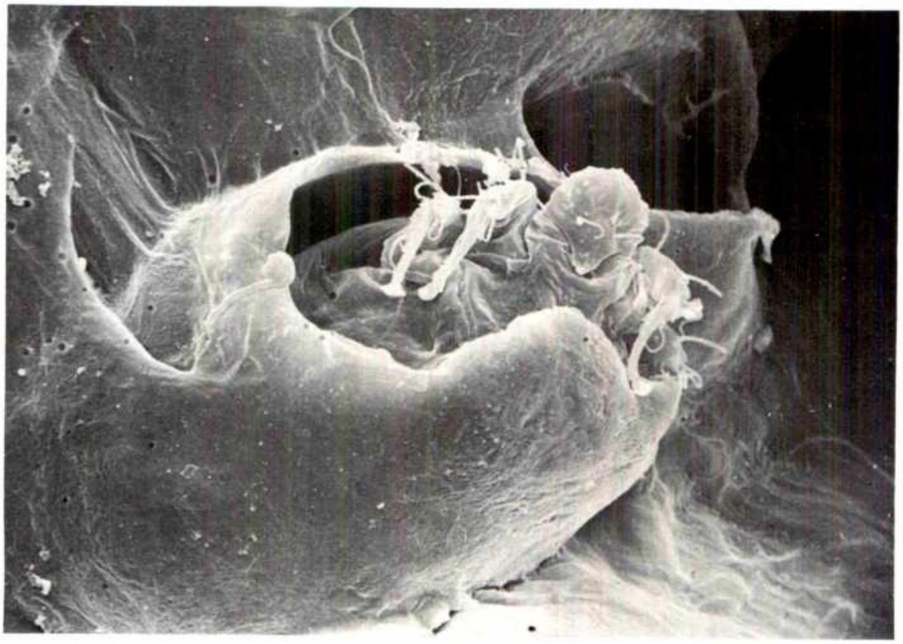

FIGURE 7. Hatching of the egg. When larval maturation has been completed, egg shells are cut out from inside. A larva is seen here to push on the free border of the egg-shell. The empty shell will be shed later on with the surrounding corneocytes, to which it seems to be anchored by means of an amorphous material.

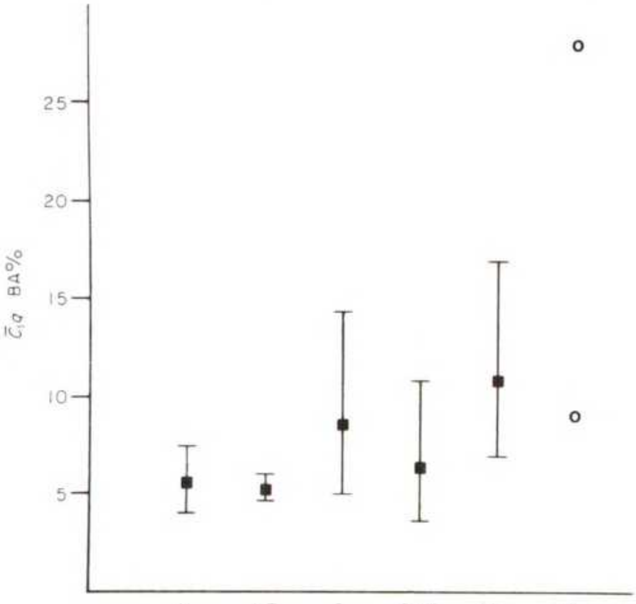

FIGURE 8. Geometric mean (± standard deviation) of the serum C1q binding activity (C1q-BA%) in controls (N; n = 52); scabies (S; n = 29) before treatment (SNT; n = 13) and after treatment (ST; n = 16) with an acaricide ointment Crotamiton (Eurax^R). As compared to N and treated controls (NT) the significant increase in the C1q-BA in S ($P < 0{\cdot}01$) reflects patients under local treatment (ST; $P < 0{\cdot}001$). The serum C1q-BA in controls was not modified after application of an acaricide ointment (N-NT; $P = 0{\cdot}7$). In hyperkeratotic scabies (HS) (circles) one point is at the upper normal limit (9%) (patient 2) and the other one is significantly increased (28%) (patient 1).

TABLE 1. ^{3}H-Thymidine flash labelling indices (%) of basal cells of the epidermis (BLI), mononuclear cells infiltrating the dermis (DLI) and peripheral blood lymphocytes (PBL-LI) in two patients with hyperkeratotic scabies are compared with figures obtained from healthy subjects (N), allergic contact dermatitis (ACD), and irritant dermatitis (ID)

	Previous studies		Scabies patient 1	Scabies patient 2
BLI	N :	4·5±0·2*	13·9	7·1
DLI	ACD :	3·7±1·3†	3·7	0·9
	ID .	0·4±0·2†		
PBL-LI				
	RFC: N :	0·42±0·36‡	3·1	2·1
	ACD :	4·95±3·57‡		
	NRFC: N :	0·14±0·12‡	2·2	3·1
	ACD :	1·60±1·87‡		

* Lachapelle (1970)
† Van Neste & Lachapelle (1981)
‡ Lachapelle *et al.* (1980)
RFC—Rosette-forming cells.
NRFC—Non-rosette-forming cells.

mite burrows deeper and reaches the granular and/or malpighian layers, disturbed keratinization results in parakeratosis, which constitutes all or part of the walls of the burrow. This process occurs quite rapidly. Indeed some parakeratotic cells are already present under the body of the mite, while eggs laid down previously are supported by a multilayered parakeratotic horny floor. Cumulative insults to the epidermis by numerous burrowing mites stimulate reactive processes such as psoriasiform hyperplasia and mixed cell infiltration of the dermis.

Although it is not possible to infer turnover time and rate from ^{3}HT flash labelling indices (Wright, 1980), one can speculate that the association of increased ^{3}HT labelling indices, epidermal hyperplasia and thinning of the suprapapillary malpighian layers, most probably reflects increased epidermal turnover. The reduced time available for completion of the life cycle of the scabies mite would result in significant limitation of the mite population. But retention of densely packed parakeratotic corneocytes favours development of numerous eggs which should, under normal corneal shedding, be lost before completion of larval maturation. Disturbed keratinization seems thus to counteract the deleterious subcorneal life conditions for the mite resulting from the host's inflammatory response.

Furthermore, activation of the immune system as suggested by increased blastogenesis of T and B cells infiltrating the dermis and circulating in the peripheral blood enhances the non-specific epidermal (Van Neste & Lachapelle, 1980) and specific-immune mediated reactive process. The reasons why this inflammatory response is not effective in limiting the parasitic population are still unclear. Interactions between antigens and antibodies or specific receptors on cell membranes do not necessarily favour the host *versus* the parasite. Indeed in our two cases of HS, the blastogenic response affects equally T and B cells in the peripheral blood. This looks very similar to the blastogenesis of circulating lymphocytes in patients with atopic dermatitis

(Lachapelle *et al.*, 1980; Van Neste & Lachapelle, 1981); in contrast, patients with widespread allergic contact dermatitis develop a predominantly T cell blastogenic response (Lachapelle *et al.*, 1980). The relative increase of B cell blastogenesis in hyperkeratotic scabies could, by analogy to our findings in atopic dermatitis, reflect defective control of B cell response by T suppressor cells. This supports the concept of B cell activation and antibody synthesis already reported in common scabies (Van Neste & Salmon, 1978; Van Neste, 1981; Falk & Bolle, 1980a,b) and confirmed here by the presence of circulating antigen-antibody complexes. Finally other factors such as 'variability' of *Sarcoptes scabiei* (Fain, 1978) also have to be integrated in a more general concept of host-parasite relationships in scabies.

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The fibrinolytic activity of human oral epithelium and epidermis

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SUMMARY

The fibrinolytic activity of normal human oral epithelium and abdominal epidermis was studied by two techniques. With fibrinolytic autography, dorsal tongue, ventral tongue, cheek, palate and gingival sulcus epithelium showed fibrinolytic activity but oral gingival epithelium and the epidermis showed no activity. With a fibrin plate technique, potassium thiocyanate extracts of epidermis and oral epithelium all showed strong fibrinolytic activity, but activity in tris buffer extracts was demonstrable only with oral epithelium, and the activity was much weaker. The fact that the tris buffer extractable fibrinolytic activator was demonstrated only in oral epithelium suggests that the presence of this activator in epithelium is related to the type of keratinization and/or its rate of cell turnover.

Plasminogen and plasminogen activators are widely distributed in circulating blood, body fluids and tissues, the effects of the spontaneous activation of plasminogen generally being controlled by the associated presence of inhibitors of both plasminogen activation and plasmin. Plasmin is able to hydrolyse several blood clotting factors and to activate both complement and Hagemann factor, in addition to its better known role in the degradation of fibrinogen and fibrin. The role of this enzyme activation system in many tissues is at present obscure, but in view of the broad activity of plasmin, it could theoretically be of importance in normal physiological function and in certain pathological processes including inflammation.

Plasminogen activators in human tissues are particularly related to the endothelial cells of systemic veins, venules and capillaries, from whence they may be released into the circulating blood. However, activators have also been demonstrated in epithelium from the endometrium (Todd, 1964), vagina (Tympanidis, King & Astrup, 1968), bladder (Lindell & Porter, 1974), cornea (Pandolfi & Astrup, 1967) and parts of the male genital tract (Kester, 1971), and a variable number of cells in buccal mucosal smears show plasminogen activator activity (Birn & Fejerskov, 1971; Wunschmann-Henderson & Astrup, 1972). Previous investigators have not been able to demonstrate fibrinolytic activity in either oral epithelium (Lucas & Tacke, 1970;

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Bjorlin, Pandolfi & Nilsson, 1971; Harris & Pannell, 1973) or epidermis (Turner & Ryan, 1969). This difference in fibrinolytic activity between isolated cells in buccal smears and whole oral epithelium could be due to activator release during cell degeneration or death, as a study of the fibrinolytic activity of explants of living and dead tissues suggested that, while activators may be synthesized by vital, metabolic cells, they may be released by cell breakdown (Astedt & Pandolfi, 1972).

When oral mucosa or skin is examined by the technique of fibrinolytic autography in which a frozen section of unfixed tissue is covered with plasminogen-rich fibrin and incubated, the lysis developing over vessels in the connective tissue may be so extensive that with longer periods of incubation it extends over the epithelium. As a result it is often impossible to determine whether or not plasminogen activators are also present in the epithelium. Two different plasminogen activators can be extracted from connective tissue (Albrechtsen, 1958): a labile one extractable with saline and a stable one more readily extractable with potassium thiocyanate. Nishioka & Ryan (1971) found no fibrinolytic activity in tris buffer extracts of epidermis, but potassium thiocyanate extracts were not investigated. Moody (1976) found that there was a marked difference in the ability of desquamated human oral epithelial cells in saliva to lyse human and bovine fibrin, lysis only being demonstrable with bovine fibrin if streptokinase was also present. Human epidermis also only causes lysis of bovine fibrin if streptokinase is present (Nishioka & Ryan, 1971), but no studies of the fibrinolytic activity of human oral epithelium or epidermis using human fibrin have been described.

It is important to determine whether or not plasminogen activators are present in oral epithelium or epidermis because, for example, the activation of complement by plasmin formation within the epithelium could be a factor in the initiation and maintenance of acute inflammation in the oral mucosa and skin. Few investigations of local fibrinolysis in skin have been reported since Turner, Kurban & Ryan (1969) commented that further investigations related to the fibrinolytic system in skin may provide information pertinent to the understanding of cutaneous pathophysiology. This paper describes the results of experiments designed to show whether or not activators of fibrinolysis are present in, or can be extracted from, normal human oral epithelium and epidermis.

MATERIALS AND METHODS

Specimens of abdominal wall skin and mucosa from the dorsal tongue, ventral tongue, cheek and palate were obtained from routine autopsy cases, and fresh gingival mucosa was obtained from surgical gingivectomy tissue. After washing in tris buffer pH 7·8, $1{\cdot}5 \times 10^{-1}$ M for 5 min, the specimens were incubated for 2 h at 37°C in 2 M sodium bromide, after which the epithelium could generally be easily stripped from the connective tissue. Occasional specimens in which epithelial separation was difficult were discarded. The separated epithelium was washed in tris buffer.

For study with the fibrin plate technique, 0·05 g specimens of epithelium were ground in 1 ml of either tris buffer or 2 M potassium thiocyanate for 5 min in a Griffith's tube homogenizer. Each suspension was centrifuged at 3500 r.p.m. for 5 min and the supernatant examined for fibrinolytic activity by the fibrin plate method using human fibrin, each extract being plated in duplicate by applying 0·03 ml drops (Southam & Moody, 1975). Ten tris buffer and ten thiocyanate extracts of each type of epithelium were examined.

For study by fibrinolytic autography, the specimens of epithelium were frozen with compressed carbon dioxide and 5 μm thick frozen sections cut on a cryostat and examined by the fibrin slide technique using human fibrin (Southam, Moody & Kowolik, 1981). Plasminogen present in the fibrin film is activated by any plasminogen activator in the tissue with subsequent lysis of the fibrin and the sites of plasminogen activator in the tissue were located as pale or clear areas in the fibrin background. Control of any non-specific proteolytic activity was achieved by incorporating 0·01 ml of 10^{-2} M epsilon aminocaproic acid (εACA) in a control film. At this concentration εACA functions as a non-competitive inhibitor of plasminogen activation (McNicol & Douglas, 1964). More than fifteen specimens of epithelium from the tongue, gingiva and skin were examined, but only three specimens of cheek and palate epithelium were examined.

RESULTS

Fibrin plates

All the potassium thiocyanate extracts produced lysis and, while some of the tris buffer extracts of oral epithelium did not produce lysis, none of the tris buffer extracts of abdominal epidermis

TABLE 1. Means and standard deviations of the average areas of lysis (in mm^2) produced by 2 × 0·03 ml drops of tris buffer and 2 M potassium thiocyanate extracts upon human fibrin plates after 19 h incubation at 37°C

		Dorsal tongue	Ventral tongue	Palate	Cheek	Skin
Tris buffer extract	Mean	98	36	36	38	0
	s.d.	38	46	47	41	0
Potassium thiocyanate extract	Mean	279	201	249	233	225
	s.d.	69	18	33	40	86

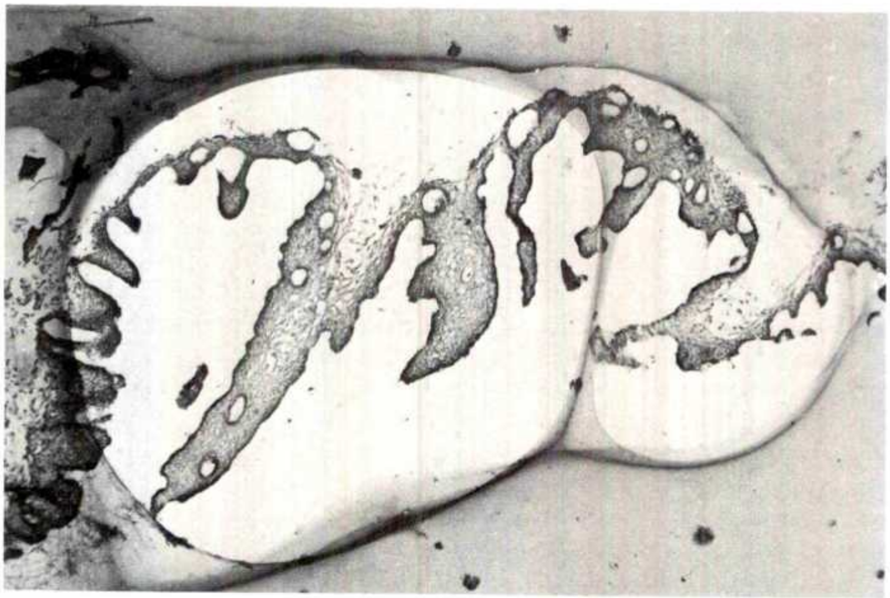

FIGURE 1. Human dorsal tongue epithelium. Fibrinolytic autograph incubated for 18 h at room temperature showing extensive foci of lysis over the epithelium (× 30).

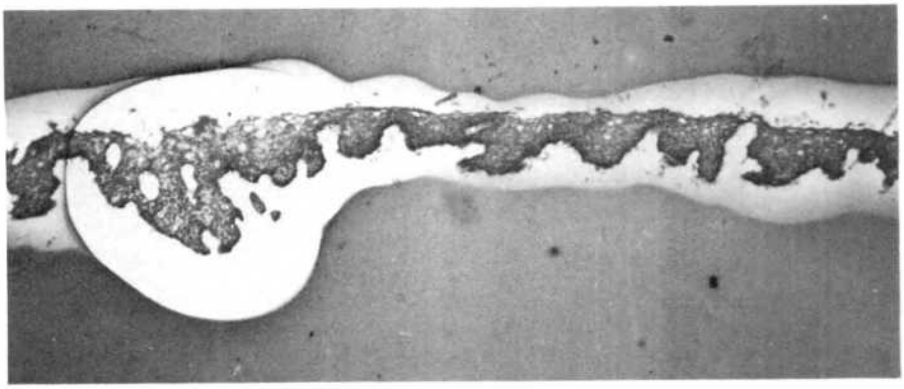

FIGURE 2. Human ventral tongue epithelium. Fibrinolytic autograph incubated for 18 h at room temperature and 1 h at 37°C showing lysis over the whole epithelium (× 30).

produced lysis. The means of the areas of lysis produced by the different extracts are given in Table 1, and it is seen that the areas of lysis produced by the thiocyanate extracts of oral epithelium were at least three times greater than the areas produced by the tris buffer extracts.

Fibrinolytic autography

Lysis developed over epithelium from the cheek, palate, dorsal tongue (Fig. 1) and ventral tongue (Fig. 2). Not all the specimens examined produced lysis and, when lysis did occur, it often varied considerably over the specimens. In most specimens the lysis was centred over the middle of the prickle cell layer, and in no specimen was lysis related to desquamating cells on the surface. Lysis never developed over gingival epithelium, except for gingival sulcus epithelium over at least part of which lysis always developed. Lysis never developed over epidermis. No lysis occurred if εACA was included in the fibrin at an end concentration of 10^{-3} M, showing that the lysis produced was due to plasminogen activation.

DISCUSSION

The fibrinolytic autography results showed that plasminogen activators are present in both keratinized and non-keratinized human oral epithelium, although no evidence for their presence was found in keratinized gingival epithelium or epidermis. Similar unpublished investigations of the fibrinolytic activity of rat oral epithelium showed a relationship between the fibrinolytic activity and the type of keratinization. Staining of keratinized oral epithelium with Mallory's connective tissue stain shows two types of orthokeratinization, one in which the whole keratin stains red (complete keratinization) and one in which only the deeper parts stain red (incomplete keratinization). In the rat, only incompletely keratinized epithelium shows fibrinolytic activity when examined by fibrinolytic autography. A similar conclusion can be made for human oral epithelium from the results of the present experiments, with incompletely keratinized dorsal tongue epithelium showing fibrinolytic activity and completely keratinized oral gingival epithelium showing no activity. Ventral tongue, cheek and gingival sulcus epithelium are non-keratinized and behaved in a manner similar to incompletely keratinized epithelium. Although human palatal epithelium may be completely keratinized, the specimens used in the present experiments were from denture wearers when the epithelium may be incompletely orthokeratinized, parakeratinized or even non-keratinized.

The fibrin plate experiments showed that plasminogen activators can be extracted from both oral epithelium and epidermis with potassium thiocyanate, the fibrinolytic activity of similar tris buffer extracts being either much reduced or absent. It has been suggested that the stable activator present in thiocyanate extracts of tissue may not be available for normal physiological processes (Albrechtsen, 1958) and this suggestion is supported by the fibrinolytic autograph experiments which showed lysis only over epithelium from which tris buffer extracts also showed fibrinolytic activity with fibrin plates.

Incompletely orthokeratinized epithelium in the rat has a higher rate of cell turnover than completely orthokeratinized epithelium and it may be the cell turnover rate which is the essential factor in determining both the type of keratin form and the amount and type of plasminogen activator (Southam *et al.*, 1981). If this hypothesis is correct, then epidermis which is not ortho-keratinized and/or has a higher than normal rate of cell turnover should show increased fibrinolytic activity. Few fibrinolytic autography studies of pathological skin have been reported, but Haustein (1969) found that spongiotic and excoriated areas of epidermis in acute and subacute eczema, as well as parakeratotic epithelium of the transitional mucosa of the inner surface of the prepuce, show fibrinolytic activity. Parakeratotic psoriatic epidermis is reported as having considerable fibrinolytic activity (Haustein, 1971; Ryan, 1976). Ryan (1980) suggested that this might be related to loss of a fibrinolytic inhibitor in psoriatic epidermis, but the accelerated epidermal turnover in psoriasis could be a more important factor.

The significance of the plasminogen activator activity of epithelium *in vivo* has yet to be determined. The results of investigations into the stickiness of cells to glass in tissue cultures indicate that most cells possess an extramembranous coating, containing protein and polysaccharide, which is associated with cellular stickiness (Schultz-Haudt *et al.*, 1963). Efforts to identify such a substance histochemically have, however, failed. Wunschmann-Henderson & Astrup (1972) suggested that epithelial fibrinolytic activity might be involved in the mechanism of cell desquamation, the activation by epithelial cells of interstitial plasminogen acting simultaneously with other lysosomal enzymes in breaking down the substances associated with cell adhesion and so enhancing cell desquamation on the surface. Ryan (1980) has similarly suggested that plasmin in epidermis should release surface antigens and encourage separation of cells.

In wound healing, the migration of the regenerating epithelium over the ingrowing granulation tissue and beneath the fibrin clot would be enhanced by the fibrinolytic activity of the epithelium. Activation of complement by plasmin within epithelium would release C3a and C5a which have a chemotactic effect on neutrophil and eosinophil leucocytes and macrophages. Large numbers of neutrophil leucocytes are often seen in inflamed oral epithelium (and psoriatic epidermis) with relatively few neutrophils in the adjacent connective tissue. The possible significance of the fibrinolytic activity of gingival sulcus epithelium in inflammatory gingival and periodontal disease has been discussed elsewhere (Southam *et al.*, 1981).

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Effect of cyproterone acetate on rate of hair growth in hirsute females

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SUMMARY

We determined by trichometry the rate of hair growth in ten hirsute patients receiving a combination of cyproterone acetate (CA) 50 mg/daily and 'Diane'® (CA 2 mg + ethinyl oestradiol 0·05 mg). In two patients there was a prompt and persistent reduction in hair growth; in three the response was less marked, and in the remaining five patients the rate of hair growth fluctuated during therapy. However, there was an overall reduction in hair growth in all but one patient during the 12 months of treatment and all patients noticed that their hair became softer, thinner and lighter in colour.

The discovery by Neumann *et al.*, (1966) that the progestagen, cyproterone acetate, had marked antiandrogenic properties was soon followed by several clinical trials of its use in the treatment of hirsutism (Hammerstein *et al.*, 1969; Ismail *et al.*, 1974; Hammerstein *et al.*, 1975; Nardi *et al.*, 1975; Dewhurst *et al.*, 1977; Schindler, 1977; Aydinlik *et al.*, 1977). All reports have been encouraging in that definite improvement in hirsutism was noted. The subject has been well reviewed by Stewart & Pochi (1978) and Burton (1979). However, with the exception of the papers by Ebling *et al.* (1977) and Anderson (1978), assessments have been subjective or, at best, semi-quantitative.

We have now determined objectively the rate of hair growth in hirsute patients on cyproterone acetate therapy, using a trichometer.

METHODS

Ten patients with hirsutism referred to the Gynaecological Endocrine Clinic at Groote Schuur Hospital were studied. All patients had normal or slightly elevated urinary 17 oxosteroid excretion, excluding major adrenal pathology. However, every patient except for three cases (Cases 4, 5 and 10) had biochemical evidence of hyperandrogenism.

Prior to commencement of treatment, three baseline measurements of the rate of growth of

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hair were taken at weekly intervals. Thereafter, treatment was instituted using cyproterone acetate 50 mg daily from the fifth to the fourteenth day of the menstrual cycle in combination with a contraceptive preparation containing cyproterone acetate 2 mg with ethinyl oestradiol 0·05 mg ('Diane') from day 5 to 25 inclusive. After a 7-day break the same treatment was repeated cyclically for 12 months during which time monthly hair growth measurements were made. Subjective appraisal of the treatment by each patient was recorded and photographs were taken at 3-monthly intervals. Specific side effects were enquired about and the patient was encouraged to report any other beneficial or adverse effects of the therapy.

The rate of hair growth was measured using a specially designed trichometer (Katz *et al.*, 1979). The trichometer was constructed in two parts (Fig. 1). A capillary tube was machined from clear Plexiglass rod. A hole was drilled up its centre and the tube was calibrated at 0·2 mm intervals up to a length of 12 mm (Fig. 2). A 'Penlite' torch was used both as a handle and as a light source. An adaptor was made from PVC material to replace the standard globe fitting and the graduated Plexiglass tube was fitted into the adaptor. The light from the torch globe is transmitted down the stem of the tube enabling easy and direct reading, preferably in a darkened room. Readability was improved by filling the bore of the tube with water. To facilitate this procedure, a vent hole, as described above, was incorporated. To further facilitate reading of the instrument, a binocular loupe (linear magnification × 2·5) was used. Reproducibility was assessed by an observer experienced in the use of the instrument (Table 1, observer A), who measured three hairs each five times (within-observer reproducibility). Between observer reproducibility was assessed by having a further two inexperienced observers (Table 1, B and C) measure the same three hairs. The mean ± s.d. hair length was 2·00 ± 0·11 mm. The overall mean ± s.d. for all three hairs measured by all three investigators was 2·12 ± 0·126 mm with an overall coefficient of variation of 5·9%.

Patients were required to shave a predesignated area 3 days prior to their first attendance. To ensure that the area for hair growth measurement could easily and reproducibly be located, a locator made from polyvinylchloride sheet, with graduated adjustable arms and a circular open disc with an internal diameter of 1 cm, was designed (Fig. 3). The midpoint of the vermilion border of the lower lip was used as the point of reference. From the graduations of the adjustable arms of the locator, the exact position of the area designated for study was recorded. At the first visit, five hairs were measured within the locator disc and 4 days later five hairs were again

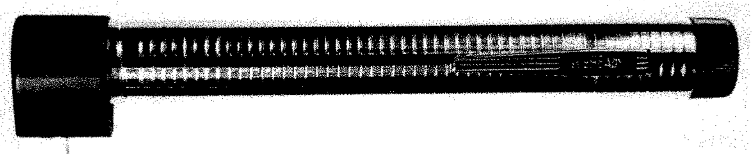

FIGURE 1. Trichometer showing Plexiglass rod fitted into a 'Penlite' torch.

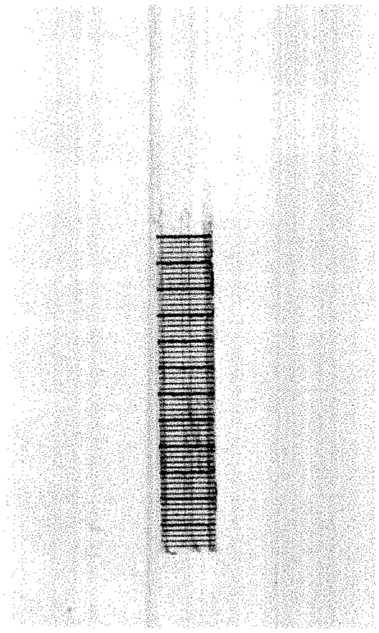

FIGURE 2. Lower segment of capillary tube showing detail of central bore and calibrations.

TABLE 1. Reproducibility of trichometric measurements: observers A, B and C measured hairs 1, 2 and 3 five times each

	Observer A (Trichometer reading in units, 0·2 mm = 1 unit)	Observer B	Observer C
	2·2	2·2	2·2
	2·2	2·0	2·4
Hair 1	2·2	2·2	2·2
	2·4	2·0	2·2
	2·2	2·0	2·2
Mean ± s.d.	2·24 ± 0·088	2·08 ± 0·11	2·24 ± 0·088
	2·2	2·0	2·0
	2·2	2·0	2·0
Hair 2	2·0	2·2	2·0
	2·0	2·0	2·0
	2·0	2·2	2·0
Mean ± s.d.	2·08 ± 0·11	2·08 ± 0·11	2·00 ± 0·00
	2·0	2·2	2·2
	2·2	2·2	2·2
Hair 3	2·0	2·4	2·2
	2·0	2·2	2·2
	2·0	2·4	2·2
Mean ± s.d.	2·04 ± 0·09	2·28 ± 0·11	2·20 ± 0·00

Overall mean ± s.d.—2·12 ± 0·126.
Coefficient of variation 5·9%.

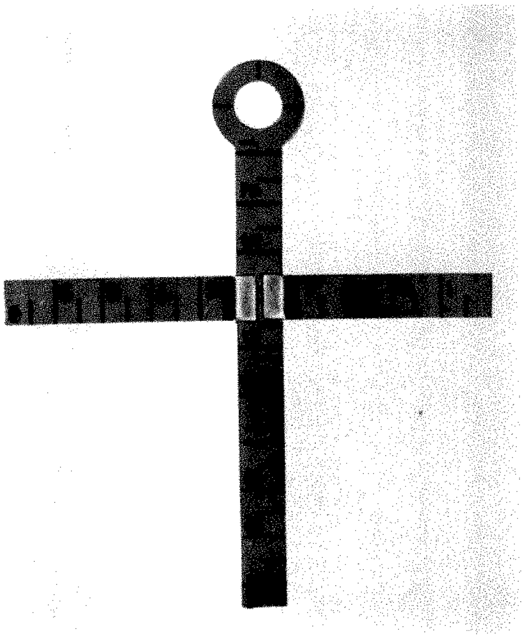

FIGURE 3. Locator used to accurately relocate the same area for hair growth measurement on subsequent visits.

measured within the same area. In seven subjects hair growth on the face was measured; the other three cases (Case nos 8, 9 and 10) had minimal facial hair and therefore an abdominal site was selected using the umbilicus as point of reference. In five patients a second site was also studied. During the 5 days of the study the patients were required to use no depilatory agents on the shaved area(s).

RESULTS

Table 2 records weekly basal hair growth rate measurements. Certain patients showed marked variability in rate of hair growth (Case nos 2 and 4), while others (Case nos 3 and 5) showed remarkable consistency from one week to the next. The mean basal rate of growth ranged from 0·20 to 0·44 mm/day in individual patients, with a group mean ± s.e.mean of 0·325 ± 0·015 mm/day.

Measurements taken during therapy for each patient are recorded in Fig. 4. All patients completed the 12-month course except Case nos 3 and 10, the latter defaulting after 8 months. Failures to attend were accounted for by valid reasons such as illness or unavoidable prior commitments. Considerable variations were seen in the response to treatment, as reflected by hair growth rate.

Only two patients (Case nos 9 and 10) showed a prompt and persistent reduction in hair growth. These patients had the highest mean basal rate of hair growth and in both the decline in rate was observed after only one month of treatment. Case 3 showed a less rapid but steadily progressive fall in growth rate, while Case 2 showed a late response, with hair growth falling significantly only after the 8th month of treatment. On the other hand, Case 6 showed a good medium term response with a fall in hair growth rate until the 10th month, when the rate

TABLE 2. Baseline rate of growth of hair (mm/day)

Case no.	Week 1	Week 2	Week 3	Mean
1	0·32	0·14	0·14	0·20
2	0·32	0·28	0·29	0·30
3	0·28	0·47	0·16	0·30
4	0·34	0·31	0·26	0·30
5	0·33	0·31	0·33	0·32
6	0·25	0·40	0·34	0·33
7	0·29	0·34	0·35	0·33
8	0·28	0·36	0·37	0·34
9	0·44	0·40	0·34	0·39
10	0·35	0·50	0·47	0·44

Mean—0·325.
S.e. mean—0·015.
Range—0·20–0·44.

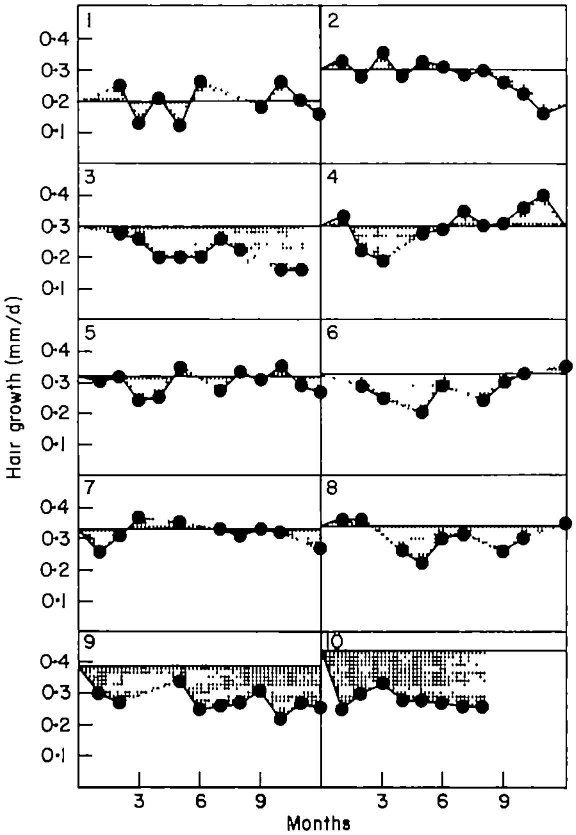

FIGURE 4. Rate of hair growth (mm/day) in ten patients treated with cyproterone acetate 50 mg daily from day 5 to 14 of their menstrual cycle with 'Diane' (ethinyl oestradiol 0·05 mg + cyproterone acetate 2 mg) from day 5 to 25 of their cycle. The horizontal line in each panel represents the mean basal hair growth rate for each patient. The dotted lines join consecutive points where intervening observations were missed.

returned to the base line, and Case 8 showed a similar response, returning to base line by the end of the study. In the other patients (Case nos 1, 4, 5, 7 and 8) the response to treatment was erratic and non-specific in that hair growth fluctuated haphazardly above and below the mean basal growth rate. The final measurement after 8–12 months of treatment was less than the mean basal value in seven out of ten cases. However, the area under the horizontal line representing overall reduction in rate of hair growth in each individual was greater than the area above this line in all but one patient (Case no. 1), and for the group as a whole this area (909 mm^2) was nine-fold greater than that above the line (108 mm^2).

In the five patients in whom the rate of hair growth was assessed at two sites, there was no statistical difference in rate of change in hair growth on face vs abdomen or on abdomen vs thigh (applying the Wilcoxon matched-pairs signed ranks test).

All patients were impressed by the change in quality of hair, even those that showed little decrease in hair growth. The hair appeared to become softer, thinner and lighter in colour and once noticed, the change persisted throughout the study period.

Only three of our patients complained of acne at the initial assessment, and in all, marked improvement was noted after only 1 month of therapy. This improvement was maintained throughout the period of study. All patients noticed a decrease in skin greasiness, particularly with regard to scalp skin. After about 4 months treatment, two patients (Case nos 2 and 10) mentioned that their scalp hair seemed to be growing 'straighter', yet two patients (Case nos 5 and 9) who had very curly hair noticed no difference. During the first 2 months of treatment side-effects were common. Only two patients reported no adverse effects. In the remaining eight, tiredness, with or without depression, was common, these symptoms being experienced maximally during the 10 days of cyproterone acetate therapy. The adverse effects wore off after approximately 3 months of treatment. An increase in appetite was another common effect and in some cases this persisted throughout the trial. However, none of the patients had difficulty maintaining their original weight.

DISCUSSION

Several clinical trials studying the effect of cyproterone acetate on hirsutes and/or acne have reported a beneficial effect. Ebling *et al.* (1977) and Anderson (1978) quantified the response by microscopic measurement of shaved hairs, but most other studies used only subjective or semi-quantitative assessment.

The present study has assessed objectively the effect of cyproterone acetate on the rate of hair growth in hirsute females. By using a simple trichometer, a binocular loupe and a locating device (Katz *et al.*, 1979) it was possible to measure the rate of hair growth accurately and reproducibly. The technique was simple, non-invasive and required no special expertise. Very fine, downy vellus-type hair was seen with difficulty and could not easily be measured but this could be overcome by the application of mascara or other colouring matter to make the hair more visible.

Basal rate of hair growth, which showed inter- and intra-patient fluctuations from week to week, ranged from 0·20 to 0·44 mm/hair/day with a mean for the group of 0·33 mm/hair/day. In a randomly selected group of females, Trotter (1924) found the mean rate of growth for leg hair to be 0·20 mm/day, while pubic and axillary hair grew at 0·30–0·31 mm/day. Male facial hair has been found to grow at a rate of 0.10–0.40 mm/day (Saitoh *et al.*, 1976) and it was interesting that the selected group of hirsute females studied by us showed an almost identical range.

The final measurement of hair growth was less than the basal measurement in seven of our ten cases, which is similar to the results reported by Dewhurst *et al.* (1977) (71%), Aydinlik *et al.*

(1977) (70%) and Braendle *et al.* (1974) (70%). However, the overall growth rate throughout the period of treatment diminished in all but one patient. The erratic fluctuations in rate of hair growth observed prior to therapy were not abolished during cyproterone acetate treatment suggesting that, while anti-androgenic therapy alters the rate of hair growth, it does not affect the basic pattern of growth.

While trichometric measurements were being made patients were encouraged to comment on any observations that they had made spontaneously. Three patients with acne noticed marked improvement within the first month of therapy while four other patients reported a dimunition in skin greasiness. These observations are in keeping with the findings of Neumann & Elge (1966) in rats, and Burton, Laschet & Shuster (1973) in men, of a decreased rate of sebum production during cyproterone acetate therapy. Within the first 3 months, most patients noticed a change in the nature of their hair in that it became finer in quality and lighter in colour. This was observed by several patients well before any measurable change in rate of hair growth. The change in hair quality was subsequently confirmed by microscopic examination of individual shaved hairs, agreeing with the findings of Ebling *et al.* (1977). Once changes in acne, skin greasiness and hair quality had been noted, the new qualities were retained throughout the treatment period.

When cyproterone acetate was used in a daily dose of 100–200 mg (Ismail *et al.*, 1974; Nardi *et al.*, 1975; Lachinsky *et al.*, 1976; Dewhurst *et al.*, 1977; Schindler, 1977) improvement in hirsutes was observed in 65–72% of cases. Our study showed that comparable results can be achieved with only 50 mg. Recently Aydinlik's multicentre trial (1977) in which only 'Diane' (cyproterone acetate 2 mg with ethinyl oestradiol 0·05 mg) was used, reported similar efficacy. It is therefore likely that the original 100–200 mg doses used to suppress libido and aggression in hypersexual males and male sexual offenders are far in excess of the doses required to effectively treat the androgenized female. We are currently objectively assessing the effect of 25 mg cyproterone acetate and the use of 'Diane' alone.

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Activity of 17β-hydroxysteroid dehydrogenase in various tissues of human skin

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SUMMARY

The activity of 17β-hydroxysteroid dehydrogenase (17β-HSD) was assayed in various tissues microdissected from the freeze-dried human skin of fourteen subjects. The apocrine sweat gland, sebaceous gland and hair follicle possessed a high activity of 17β-HSD. The enzyme activity was negligible in the epidermis, except that the scalp epidermis showed much the same activity as the hair follicle. The dermis showed variable activity because of contamination with other components.

The skin has been shown to actively metabolize testosterone into androstenedione (Δ^4) (formed by 17β-HSD) and dihydrotestosterone (DHT) and other 5α-reduced steroids (Sharp, Hay & Hodgins, 1976; Hay, 1977). Testosterone is first converted into either DHT, which is a more potent androgen than testosterone itself, or Δ^4 which is generally considered less active than testosterone. Thus, the action of androgen appears to be controlled, at least partly, by the relative strength of these two metabolic reactions.

With the aid of a collagenase treatment or microdissection technique, recent work has shown that the activity of 5α-reductase is mainly located in the apocrine and sebaceous glands in human skin except for genital skin (Hay & Hodgins, 1978; Takayasu *et al.*, 1980). On the other hand, the distribution of 17β-HSD is not fully understood: early histochemical studies demonstrated its activity exclusively in sebaceous glands (Cálman *et al.*, 1970) and several groups detected the enzyme in plucked hair follicles (Sansone-Bazzano, Reisner & Bazzano, 1972; Schweikert & Wilson, 1974), while Hay & Hodgins (1978) reported that it is more evenly located than 5α-reductase. Using our micromethod (Takayasu *et al.*, 1980), we have now shown that 17β-HSD is chiefly located in apocrine sweat glands, sebaceous glands and hair follicles.

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MATERIALS AND METHODS

Preparation of freeze-dried tissues

Skin specimens were obtained at surgery from the various regions (see Table 2.) of eight men and six women (9–73 years of age), and were treated as previously described (Takayasu *et al.*, 1980). Briefly, they were immediately frozen in dry ice, cut into sections of 30 μm thickness at −30°C in a cryostat, and vacuum-dried overnight. The sections were dissected into separate components under a stereoscope, magnification × 40, and weighed on a microelectric balance.

Incubation

1,2-^{3}H Testosterone (SA 42 Ci/mM, New England Nuclear Corp.) was purifed by thin-layer chromatography before use with a suitable amount of unlabelled testosterone. The standard incubation mixture consisted of 1 μM (5×10^5 d.p.m.) ^{3}H-testosterone, 2mM NAD, 100 mM tris-HCl buffer, pH 8·5, and 10% propylene glycol, in a total volume of 50 μl. Each tube contained 20–60 μg of dissected tissues. Incubation was carried out at 37°C for 1 h.

Extraction and isolation of steroid metabolites

After incubation, steroids were extracted and isolated by thin-layer chromatography as previously reported (Gomez & Hsia, 1968). Eluted samples were examined for radioactivity with a liquid scintillation spectrometer and the purity of Δ^4 was checked by recrystallization to constant specific activity. The activity of 17β-HSD was expressed by the amount of Δ^4 formed, since Δ^4 was the only 17-oxosteroid detected in the incubate. Blank containing only the incubation mixture was run in each experiment.

RESULTS

Effect of freeze-drying

The possible effect of freeze-drying on the 17β-HSD was studied with a specimen of axilla (from a 26-year-old male). When the homogenate of fresh whole skin was used instead of the freeze-dried sections, the activity of 17β-HSD was 64·2 pmol/mg wet weight/h. Since the dry weight of tissues is nearly 30% of the wet weight, this activity is considered equivalent to about 190 pmol/mg dry weight/h. On the other hand, the activity of the freeze-dried sections was 160 pmol/mg dry weight/h. Therefore the activity of 17β-HSD does not appear to be affected significantly by freeze-drying.

TABLE 1. Pyridine nucleotide requirements of 17β-oxidation of testosterone by human sebaceous glands. Sebaceous glands microdissected from a specimen of nevus sebaceus of a 16-year-old girl were used as the enzyme source. This also applies to Figs 1–4. Each value represents a mean and a standard error of three determinations

	NAD		NADP		
	0·2 mM	2·0 mM	0·2 mM	2·0 mM	None
Specific activity (pmol/mg/h)	61·0 ± 6·3	167·6 ± 20·7	18·1 ± 1·8	19·9 ± 2·1	4·4 ± 0·2

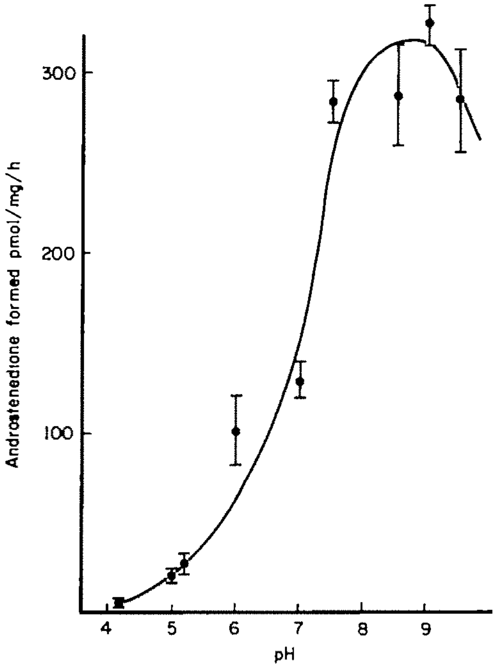

FIGURE 1. The effect of pH on the 17β-oxidation of testosterone. The experimental conditions are described in the text except for tris-HCl buffer at pH 7·5–9 5 and tris-maleate buffer at pH 4–7. Each point indicates a mean and a standard error of three determinations.

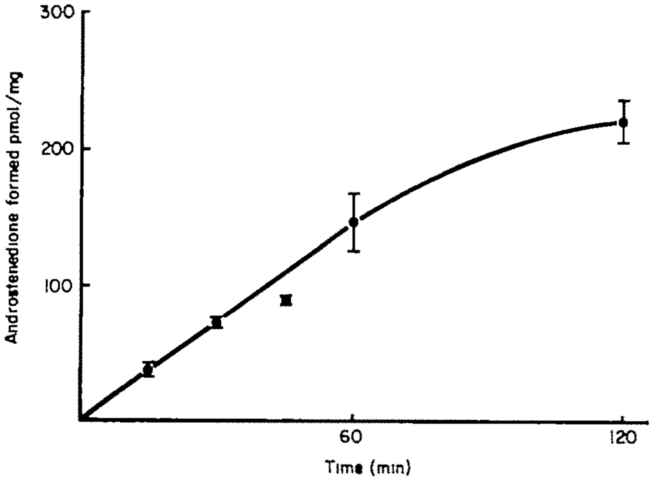

FIGURE 2. Time-course change in the formation of androstenedione from ^{3}H-testosterone by sebaceous glands. Each point represents a mean and a standard error of three determinations.

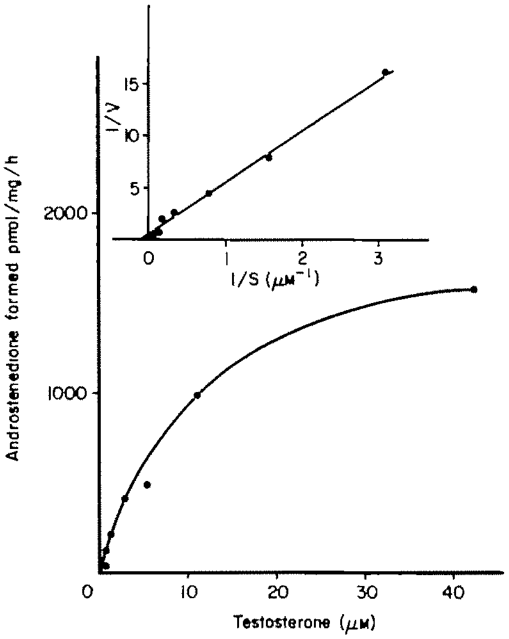

FIGURE 3. Relation between the formation of androstenedione by human sebaceous glands and the concentration of testosterone. Each point is a mean of three determinations.

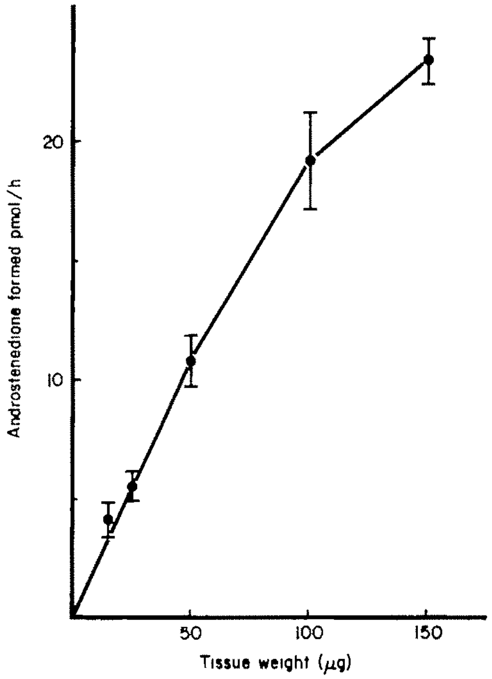

FIGURE 4. Testosterone metabolism as a function of tissue weight. Each point indicates a mean and a standard error of three determinations.

Establishment of routine enzyme assay procedure

The total recovery of radioactivity in starting material and metabolites was always greater than 80%. As a result of recrystallization, the purity of the radioactivity tentatively identified as Δ^4 was 75–95%. The routine assay procedure was established from the result demonstrated in Table 1 and Figs 1–4. Sebaceous glands microdissected from a tissue specimen of nevus sebaceus of a 16-year-old girl were used in order to establish an optimal condition, merely because it could provide an ample amount of dissected tissues. The reaction requires NAD (Table 1). The pH optimum is about 8·5 (Fig. 1). The formation of Δ^4 linearly increased to 1 h (Fig. 2). The relation between the concentration of substrate and the rate of formation of Δ^4 is given in Fig. 3. The reaction reached a plateau when the concentration of testosterone was about 4×10^{-5} M. The apparent Michaelis constant was about $9{\cdot}1 \times 10^{-6}$ M as estimated from Lineweaver-Burk plots, though the value is only a rough guide because of very small intercepts. Figure 4 displays the relationship between the tissue weight and the rate of formation of Δ^4. The rate increased linearly with less than 100 μg of tissue. Therefore, routine assay was performed on 20–60 μg of microdissected tissues incubated with 1 μM ^{3}H-1,2-testosterone for 30 min at 37°C.

17β-HSD activity of various skin tissues

The activities of 17β-HSD determined in various microdissected skin tissues obtained from sixteen individuals are summarized in Table 2. The results are expressed on a dry weight basis. The 17β-HSD activity of the sebaceous gland ranged from 111 to 426 pmol/mg/h. The activity of the sebaceous gland of the forehead or scalp, where the gland develops by the effect of androgen, does not differ significantly from that of the sebaceous gland of any other region. The apocrine gland of axillary skin showed a higher activity even than the sebaceous gland. The activity of the hair follicle was nearly one half of the value of the sebaceous gland. The age or sex does not seem to affect the enzyme activity. The activity was negligible in the epidermis, except that the epidermis of the scalp or of the forehead presented much the same activity as the hair follicle. Sometimes the dermis actively converted testosterone to Δ^4, but the activity gave a wide variation. This may be due to unavoidable contamination with different sorts of cellular components such as blood vessels, residual sweat glands, and pilosebaceous units.

TABLE 2. The activity of 17β-HSD in various tissues microdissected from freeze-dried skin. Each value represents a mean and a standard error. The figure in parentheses shows the number of samples examined

Tissue	Region	No. of subjects	Age	17β-HSD activity pmol/mg/h
Sebaceous gland	Scalp, face	7	9–73	211 ± 17·3 (26)
Sebaceous gland	Other regions*	4	26–53	280 ± 59·2 (7)
Apocrine sweat gland	Axilla	2	22,26	305 ± 25·3 (8)
Hair follicle	Scalp, axilla	7	9–46	145 ± 14·7 (14)
Epidermis	Scalp, face	7	9–42	139 ± 15·4 (19)
Epidermis	Other regions†	9	18–53	6·7 ± 1·6 (29)
Dermis	Scalp, arm, prepuce	4	18–36	19·5 ± 6·6 (10)

* Including back, axilla and prepuce.

† Including back, chest, axilla, abdomen, inguinal area, prepuce and forearm.

DISCUSSION

In the present study, the characteristics of 17β-HSD, such as Km and pH optimum, in the isolated sebaceous glands are similar to those of the enzyme in the microsomal preparations made from the skin of newborn rats (Davis, Rampini & Hsia, 1972). The preference for NAD as the coenzyme is also similar to that of rat skin enzyme.

Our present work clearly demonstrated that 17β-HSD is mainly located in the androgen target tissues of the skin such as sebaceous glands, apocrine sweat glands, and hair follicles. The activity of 17β-HSD determined in the present study and that of 5α-reductase in our previous work (Takayasu *et al.*, 1980) was high in apocrine sweat glands and sebaceous glands. These results are generally in agreement with the observation of Hay & Hodgins (1978) that 17β-oxidation was as active as 5α-reduction in these tissues. In hair follicles, the rate of 17β-oxidation is high compared with that of 5α-reduction. This is consistent with the outcome of *in vitro* studies with plucked hair follicles that Δ^4 was a major metabolite of testosterone (Fazekas & Sandor, 1972; Sansone-Bazzano *et al.* 1972; Schweikert & Wilson, 1974). Such active 17β-oxidation of testosterone in these skin appendages is in contrast to predominant 5α-reduction in other target organs like prostate (Shimazaki *et al.*, 1965), and may play some role in androgen action in the skin. For instance, Δ^4 is shown to inhibit the conversion of testosterone to DHT (Voigt, Fernandez & Hsia, 1970). Besides, it is generally accepted that testosterone metabolism is affected by the availability of cofactors such as NAD and NADPH. Thus, these two pathways are thought to affect each other with change in substrate availability and regulate the action of androgen in the skin. The epidermis of the scalp and of the face gave as high a 17β-HSD activity as the hair follicle. This may account for the predominant 17β-oxidation of testosterone in these areas of the skin compared with axillary skin (Hay & Hodgins, 1973), although its physiological significance is yet to be clarified.

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The rate of sebum excretion in man. A study on the reproducibility and the accuracy of the gravimetric method

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SUMMARY

The gravimetric method used by Strauss & Pochi (1961) to measure sebum excretion rate (SER) was modified by Cunliffe & Shuster (1969) to improve its accuracy and reproducibility. We have confirmed that their modification gives accurate and reproducible results, and a re-analysis of their data has shown that the SER in males varies slightly from week to week.

There is at present renewed interest in the use of antiandrogens for the treatment of acne and thus there is a need to investigate the effect of these drugs on sebum production. The gravimetric method for measuring sebum excretion rate (SER) was described by Strauss & Pochi (1961) and was improved by Cunliffe & Shuster (1969). The method relies on the absorption by absorbent papers of the sebum excreted from pilo-sebaceous units in a given area (6 cm^2) during a given period of time (3 h).

Despite the fact that it is a time consuming method, we have selected it because it is claimed to be accurate and reproducible (Cunliffe & Shuster, 1969). The photometric technique as described by Schaefer & Kuhn-Bussius (1970) is less accurate at high levels of sebum production.

The aim of our study was to investigate the claimed accuracy and reproducibility of the gravimetric technique.

MATERIAL AND METHODS

We followed exactly the same technique as that of Cunliffe & Shuster (1969) down to the smallest details.

We performed the test twice on twenty-eight male subjects, aged 18–35 years. Some were

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seborrhoeic and others non-seborrhoeic. No subject was on any treatment known to affect SER throughout the tests or for 2 months before.

The lapse of time between the two measurements varied from 2 to 388 days (Table 1). In all patients the SER on both sides of the forehead was determined on each occasion.

RESULTS

Accuracy of the method

We measured the SER (Table 1) on the left and right sides for each subject. A study of the possible correlation between the two values allowed us to check the accuracy of the method. This analysis showed that the left-right correlation was significant ($r = 0{\cdot}86$; $P < 10^{-4}$) (Fig. 1).

Reproducibility of the method

To study the reproducibility of the method, we measured the SER on two different days. Statistical analysis of these data demonstrates that there is no significant correlation ($r = 0{\cdot}15$) between the variation of the two SERs and the time elapsed between the two measurements (Fig. 2).

TABLE 1. Mean of sebum excretion rate values ($\mu g/cm^2/min$) in twenty-eight subjects. The mean lapse of time between measure 1 and 2 was 105 days (range 2–388)

Measure 1		Measure 2	
Left	Right	Left	Right
1·51 ± 0·48	1·55 ± 0·55	1·52 ± 0·54	1·50 ± 0·52

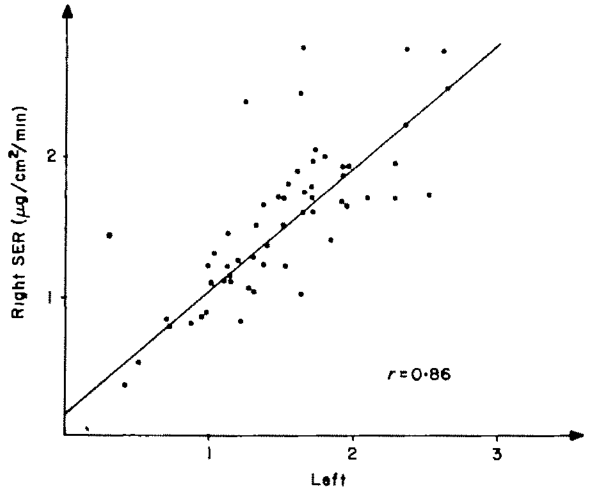

FIGURE 1. Correlation between left and right sides.

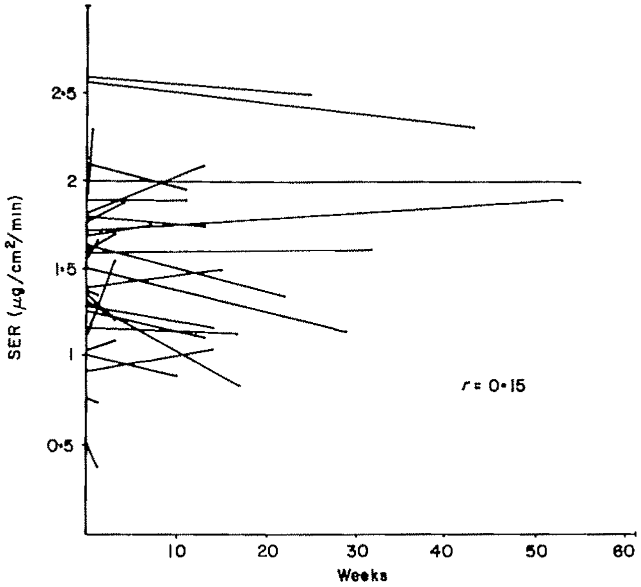

FIGURE 2. Variation of SER with time (each value is the mean of the right and left sides).

DISCUSSION

In a given subject, the forehead sebum excretion rate should be the same on each side, and with the ideal method, the correlation between left and right would be perfect ($r = 1$). In our study the left and right values are not identical for each subject, but the left-right correlation is significant ($r = 0{\cdot}86$) implying that the method is quite accurate. This accuracy is close to that we have calculated using the data of Cunliffe & Shuster (1969) ($r = 0{\cdot}89$; $P < 10^{-5}$).

We observed little difference between the mean of the SERs on both sides of the forehead and for the two measurements, whatever the level of sebum production. From previous studies and in this study the range of SER is extensive ($F = 12$; $P < 10^{-3}$), and that is why in our calculations we did not use the gross rates of SER but took into account only the variation between measurements.

To make the method as rigorous as possible, we tried to eliminate any possible source of error. The quality of the paper is very important. It should be absorbent and non-fluffy. The same grade of paper was always used for the tests (Special Velin Tissue, non-fluff, Kogh-Light Lab., U.K.) (Cunliffe, Williams & Tan, 1975). The papers were left to soak for 3 h in ether to eliminate all fatty substances prior to the tests.

The ambient temperature should be constant. The subject must be seated and quiet. He must not take food, alcohol or hot drink in order to avoid changes of the skin forehead temperature. Cunliffe, Burton & Shuster (1970) have demonstrated that a change of only one degree in forehead skin temperature provoked a 10% variation of the SER.

Only male subjects were selected to eliminate possible variations reported in women during their menstrual cycle (Katz & Scott, 1956; Burton, Cartlidge & Shuster, 1972). Tests were always performed at the same hour to eliminate circadian variations of seborrhea (Burton, Cunliffe & Shuster, 1970; Cotterill, Cunliffe & Williamson, 1973). Finally, the same technician (F.Z.) performed all the tests.

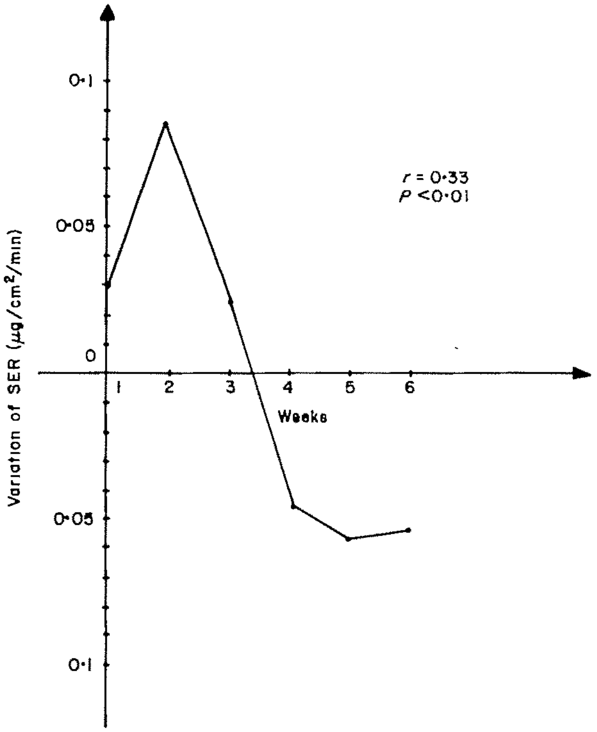

FIGURE 3. Variation of SER with time using the data of Cunliffe & Shuster (1969).

In their original study, Cunliffe and Shuster made one measurement each week for 7 weeks on ten subjects. We have re-analysed their data and the mean of the variations between the various SERs in relation to time is shown in Fig. 3. The curve is complex. It is probably a sinusoid and statistical analysis shows a low correlation ($r = 0{\cdot}33$, $P < 0{\cdot}01$) between the sebum excretion rate and repeated measurement over a period of seven weeks. Sebum excretion rate therefore varied in relation with time, contrary to what was observed in our study. We did not observe a similar curve in our study, probably because we took only two observations and this reduced the risk of hitting the crest or the trough by the excretion rate. It appears that just as there are hourly variations of seborrhea (Burton *et al.*, 1970), there is also a week to week variation (Cunliffe & Shuster, 1969). However, the variation of SER as reanalysed from the data of Cunliffe & Shuster (1969) is small compared with the variations seen between differing acne grades and following effective treatment with antiandrogens.

It was thought that the sebaceous gland in males was maximally stimulated (Strauss, Kligman & Pochi, 1962), but it is now known that, at least with pharmacological doses of testosterone, this is not so (Pochi & Strauss, 1974). A detailed time course study with small doses of testosterone may demonstrate that even physiological variations in androgen levels can influence SER in man. There are cyclical variations in the metabolism of adrenal and testicular androgens (Rowe *et al.*, 1974), and this may explain the sinusoidal variation in SER in males.

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Case Reports

Prurigo pigmentosa

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SUMMARY

We present two cases of prurigo pigmentosa, a little-known dermatosis characterized by itchy red papules which coalesce to give a reticular pattern evolving into a reticulate hyperpigmented mottling. The histological features are non-specific and some patients with this disorder respond dramatically to dapsone.

Prurigo pigmentosa is a disorder of unknown aetiology which has not been described in either the European or American literature, yet more than forty cases have been described in Japan since 1971. Recently Nagashima (1978) reviewed fourteen cases and found the disorder was more common in adult females, with an onset most often in the spring and summer months. Nagashima thought that physical trauma and possibly friction from clothes may be important in the pathogenesis and for the exacerbation of the disorder in some patients. The characteristic lesions are found mainly on the neck and on the trunk and initially present as itchy, slightly raised, red papules, occasionally coalescing to give a reticular pattern. Later, the red papules are superseded by a peculiar reticular hyperpigmented mottling. The histological appearances are non-specific but often somewhat lichenoid with inter- and intracellular oedema, oedema of the upper dermis, liquefaction degeneration of the basal cell layer, perivascular round cell infiltrate and some pigmentary incontinence. Occasional blood eosinophilia was also seen. Both itching and rash failed to respond to a wide range of treatment including oral antihistamines and topical steroids. However, oral therapy with dapsone led to a dramatic improvement in four out of fourteen cases reviewed by Nagashima (1978).

We here describe two further patients.

PATIENTS AND METHODS

Case 1

A woman aged 23 years presented in 1978 with an 18-month history of an itchy papular eruption with reticular mottling over both sides of the neck. Trichlorophenol (TCP) had been used to cleanse the affected areas for a corresponding length of time.

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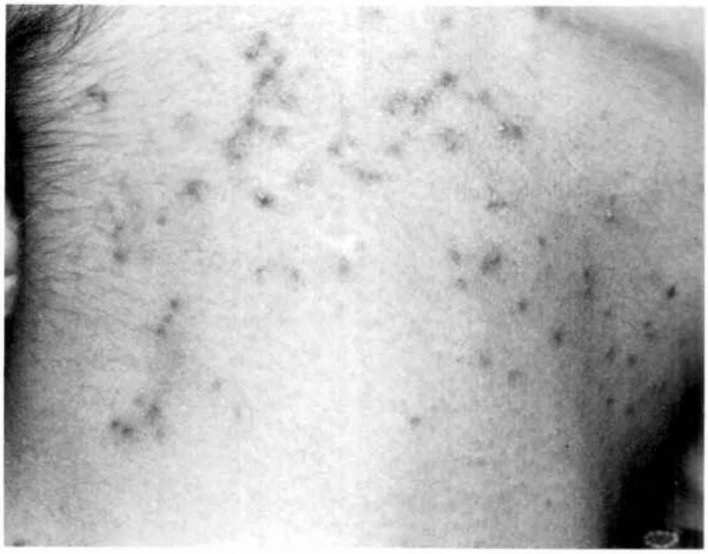

FIGURE 1. Papular prurigo, showing the characteristic papular lesions on the neck coalescing in places to give a reticular pattern.

Examination showed reddish papules, some excoriated, and marked reticular hyperpigmented mottling (Figs 1 and 2).

Haematological and biochemical investigations were unremarkable. A skin biopsy of the affected area showed a small focus of epidermal thinning, marked parakeratosis, focal spongiosis, dermal papillary oedema, dilatation of superficial blood vessels and perivascular lymphocyte cuffing. Moderate numbers of melanin laden macrophages and a few mast cells were also seen.

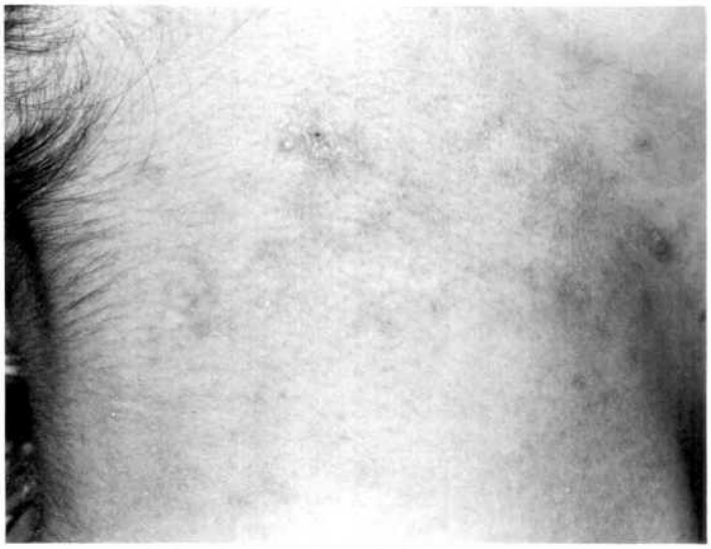

FIGURE 2. Papular prurigo, showing the curious reticular hyperpigmentation remaining in the skin after 2 weeks' treatment with dapsone.

Immunohistology of involved and uninvolved skin was within normal limits. Patch tests to the standard European series of allergens were negative.

Initially treatment was attempted using local steroids, local antiseptic agents, benzoyl peroxide and oral tetracycline, all to no avail. The rash did not disappear following discontinuation of the use of TCP, but regressed within 1 week on dapsone 100 mg daily. The rash promptly reappeared 2 weeks after the dapsone was stopped. A further course of dapsone was given for 3 months and cessation of therapy after this second course has not led to a recurrence of the eruption so far.

Case 2

A man aged 61 presented with a generalized itchy papular eruption on the trunk with a curious reticulate hyperpigmentation of five months' duration. A history of TCP usage on skin of similar duration was also elicited. He had been a heavy smoker for years.

Examination findings included finger clubbing, eczema craquelé of the back, excoriations over his neck and a reddish papular eruption over the trunk, coalescing in places to produce a reticulate pattern of hyperpigmentary change, particularly marked on the lateral aspects of the chest. Investigations were unremarkable, apart from a chest radiograph that showed increased basal markings consistent with early bronchiectasis.

Skin biopsy of affected area showed slight hyperkeratosis with a slightly pigmentated basal layer. The superficial dermis had moderate to severe lymphoid and histiocytic infiltrate around blood vessels and sweat glands. Special staining showed no increase in mast cells. Immunohistology showed no abnormal findings.

The patient was dissuaded from TCP usage and treated with hydroxyzine chloride orally and betamethasone valerate 0·1% diluted 1:4 in emulsifying ointment topically. The itching and papular eruption, along with the pigmentation, had almost disappeared after 6 months of therapy.

DISCUSSION

The two patients described have all the clinical features of prurigo pigmentosa, namely itchy red papules and a curious reticulate hyperpigmentation. The histological findings in our patients were nonspecific and neither biopsy showed any evidence of liquefaction degeneration of the basal cell layer. It has been suggested that prurigo pigmentosa may represent an unusual form of dermatitis herpetiformis (Dobson, 1979). However, immunohistology of both involved and normal skin was negative in both cases and this is strong evidence against this hypothesis. Moreover, it is unlikely that prurigo pigmentosa is a variant of lichen planus because not only are the clinical features atypical, but both the histology and immunohistology show none of the features of this disorder.

The role of trichlorophenol (TCP) in the pathogenesis of the eruption in the patients described above is problematical. Both patients had used TCP fairly energetically but there was no clinical improvement in their eruption on stopping this preparation topically. Moreover, phenolic compounds produce depigmentation of skin much more commonly than hyperpigmentation (Kahn, 1970).

The response of the first patient to dapsone was dramatic and, indeed, it was difficult to persuade her to stop this drug. Prurigo pigmentosa must join the increasing number of dermatoses which seem to respond sometimes to dapsone.

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X-linked recessive ichthyosis in three sisters: evidence for homozygosity

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SUMMARY

A family with ichthyosis, severely affecting both men and women, is reported. The clinical, histological and genetic data are strongly suggestive of X-linked recessive ichthyosis. Assay of steroid sulphatase in cultured skin fibroblasts from two ichthyotic female patients revealed an absence of this enzyme and thus confirmed this diagnosis. To our knowledge, this is the first report of proven X-linked recessive ichthyosis in women.

X-linked recessive ichthyosis is the second most common ichthyosiform dermatosis after autosomal dominant ichthyosis vulgaris. Its prevalence has been estimated at approximately 1 in 6000 males in England (Wells & Kerr, 1966a) but varies in different populations (Ziprkowski & Feinstein, 1972).

We are reporting a family with X-linked recessive ichthyosis in which not only males but also three female members are severely affected (Fig. 1).

CASE REPORTS

Cases II-1, II-2 and III-7 were available for clinical and histopathological examination (Fig. 1).

Case II-1

This 53-year-old white woman was born of consanguineous parents. She has suffered from a severe scaling, non-pruritic dermatosis present since birth. In winter-time the lesions become worse, but over the years there has been some improvement in their overall severity. On examination most of the body surface is covered with light to dark grey scales, the largest being on the legs and measuring about 12 × 16 mm. There is no underlying erythema or any keratosis pilaris. The most severely affected areas are the upper and lower extremities (Fig. 2a), the back,

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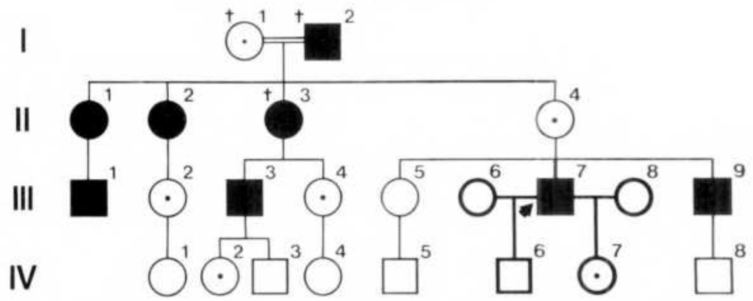

FIGURE 1. Reported family with X-linked recessive ichthyosis. Cases II-1, II-2 and III-7 were examined. Assay of steroid sulphatase activity in cultured skin fibroblasts from female patients II-1 and II-2 revealed an absence of this enzyme. The mothers of I-1 and I-2 were sisters. Ichthyosis—■●; deceased—†; obligate carrier—⊙.

buttocks and scalp. The only normal appearing areas are the palms and soles (Fig. 2b), flexural creases, anterior aspect of the neck, and the face except for the cheeks which are slightly involved. There are no other clinically detectable abnormalities.

The patient had one miscarriage and was delivered of an ichthyotic child by Caesarean section. At the age of 43 she had a right mastectomy for carcinoma and a hysterectomy for reasons unknown to her.

Case II-2

This 57-year-old woman is one of the three sisters of Case II-1. The history of her scaling dermatosis is the same as in Case II-1 except that there has been no overall improvement with age. Her clinical picture is very similar to that of her affected sister but differs in the following

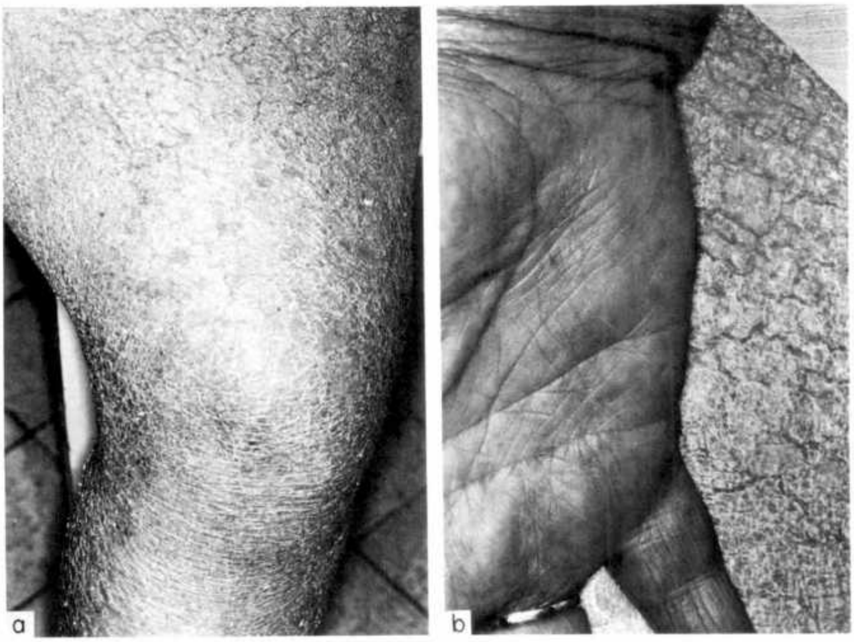

FIGURE 2. Ichthyotic woman II-1. (a) Severe involvement of lower extremity with large scales. (b) Normal palm against background of pronounced scaling of right thigh.

respects. The neck, popliteal and antecubital folds are slightly affected. The palms and soles are normal except for an area with increased skin markings in the middle of the soles. The finger nails show minor irregularities. There are no other clinically detectable abnormalities.

The patient had one normal pregnancy and no miscarriages. She has enjoyed good general health except for episodes of mental depression.

Case III-7

This 27-year-old man is the nephew of Cases II-1 and II-2. His scaling dermatosis has also been present since birth. Besides seasonal variations it has shown some overall improvement since the age of 19 years. The characteristics of his prominent generalized scaling (Fig. 3) are close to those of his maternal aunts. His palms and soles are the only sites free of lesions, and all his flexural creases are slightly affected. There are no other clinically detectable abnormalities although he has been under treatment for epileptic fits.

In none of the above described cases have there been complaints attributable to insufficient perspiration.

Histopathology

Skin biopsies were taken from the right arm in Cases II-1 and II-2 and the left forearm in Case III-7. All biopsy sites were severely affected. The microscopic picture was practically identical in Cases II-1 and II-2 (Fig. 4a, b). There was pronounced orthokeratotic hyperkeratosis. While in some regions the granular layer was composed of two and sometimes three cell layers, in other regions, on the same section, it was one cell-layered and poorly visible. The Malpighian layer was atrophic. In the male patient III-7 (Fig. 4c), in addition to the pronounced orthokeratotic hyperkeratosis the granular layer stood out well and was composed mostly of two to three cell layers. The epidermis was somewhat hyperplastic.

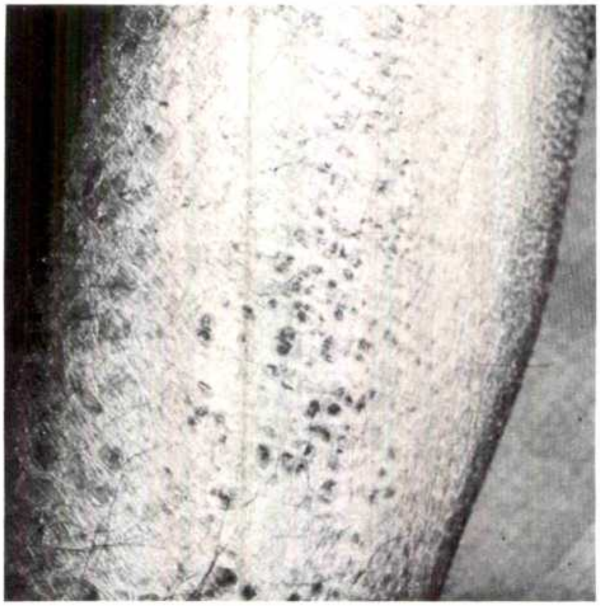

FIGURE 3. Ichthyotic man III-7. Prominent scaling on forearm.

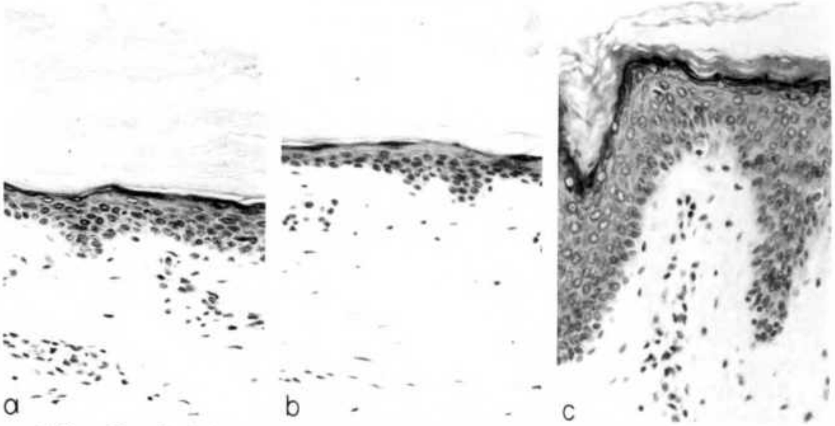

FIGURE 4. (a) Case II-1. Orthokeratotic hyperkeratosis, one to two cell-layered granular zone and atrophic Malpighian layer. (b) Case II-2. Same features except for reduced granular layer. (c) Case III-7. Orthokeratotic hyperkeratosis with a granular layer that stands out well. All sections were stained with H & E (× 160).

There were no other significant changes or any observable increase in mitotic figures in any of the biopsy specimens.

Steroid sulphatase activity

Additional skin biopsies were taken from Cases II-1 and II-2. Growth of fibroblast cultures and assay of steroid sulphatase activity were done essentially as described (Hameister *et al.*, 1979) using tritiated dehydroepiandrosterone sulphate as substrate. The results are given in Table 1: steroid sulphatase activity was absent in both patients.

Determination of the karyotype and electron microscopy could not be done, and the patients refused to have an ophthalmological examination.

The pedigree

The pedigree (Fig. 1) comprises five affected males and three affected females. One male (III-7) and two females (II-1 and II-2) have been examined, three other males (III-1, III-3 and III-9) are living and one female (II-3) and one male (I-2) are deceased. Allegedly the ichthyosis of the

TABLE 1. Steroid sulphatase activity in X-linked recessive ichthyosis patients, heterozygotes, and controls

	pmol DHEAS* cleared per mg protein and hour	
Cases II-1 and II-2 (homozygotes)	< 5	
Male patients (hemizygotes)	< 5	
Heterozygotes (n = 5)	375 ± 129	(mean ± 1 s.d.)
Male controls (n = 10)	419 ± 99	(mean ± 1 s.d.)
Female controls (n = 10)	713 ± 116	(mean ± 1 s.d.)

* Assay of steroid sulphatase activity in skin fibroblast cultures was done essentially as described (Hameister *et al.*, 1979) using tritiated dehydroepiandrosterone (DHEAS) sulphate as a substrate.

unexamined and deceased subjects was of the same type and severity as that of the examined cases. The cause of death of I-2 could not be determined; his daughter II-3 died of cerebral haemorrhage at the age of 56.

Subject I-2 was married to a 1st degree cousin (I-1) whose skin was normal; their mothers were sisters. There have been no other known (or admitted) cases of consanguinity, and none of the descendents of I-2 has been married to an individual with ichthyosis or a family history of ichthyosis. There have been no manifestations of atopy in the family.

Case II-1 is the only woman to have had a Caesarean section. We could not determine for any of the other females whether there had been any post-term deliveries or other labour difficulties.

DISCUSSION

From the point of view of gross morphology, the three reported patients with ichthyosis call for a differential diagnosis between X-linked recessive ichthyosis (XRI), lamellar ichthyosis (LI) and even a severe form of autosomal dominant ichthyosis vulgaris (ADI). The clinical differentiation between ADI and XRI has been outlined by Wells & Kerr (1966a). From this and other clinical studies (Merrett *et al.*, 1967; Ziprkowski & Feinstein, 1972) it would appear that follicular hyperkeratosis and palmo-plantar involvement are absolute differentiating criteria, in so far as they have never been reported in XRI but occur frequently in ADI. None of our patients has follicular hyperkeratosis or palmo-plantar lesions except for Case II-2 who has increased plantar markings in the middle of her soles. In our experience such minimal palmar or plantar involvement may rarely occur in males with proven XRI. Sometimes it may be difficult to distinguish clinically XRI from LI. In LI the flexor surfaces tend to be sites of predilection (Schnyder, 1970) and ectropion of the eye lids and hyperkeratosis of the palms and soles are frequent findings (Frost, 1973). In our patients the flexural creases are either free or minimally affected; they have no ectropion and only one of them has slight plantar involvement.

The histopathological differences between XRI and ADI have been pointed out by several authors (Wells & Kerr, 1966b; Fräki, Kuokkanen & Hopsu-Havu, 1973). Cases II-1 and II-2 do not show a homogeneous microscopic picture. On the same section, some regions are compatible with XRI while other regions are suggestive of ADI. In our experience, such a variation can be encountered in proven cases of XRI, particularly if one examines large biopsy specimens. The histopathology of Case III-7 corresponds to the picture for XRI as described in the literature. It should be pointed out, however, that unless histological sections are specifically examined for the number of mitoses it may be impossible to distinguish between XRI and LI (Frost, 1973). Such specific counting of mitoses was not done.

The pedigree (Fig. 1) suggests three possible modes of inheritance. An autosomal dominant trait is possible because of numerous examples of obligate carriers with clinically normal skin in ADI (Kuokkanen, 1969). An autosomal recessive trait would call for multiple consanguineous marriages; subjects I-1 and I-2 were first cousins but other cases of consanguinity are denied by the patients and are unlikely. Actually, the mode of transmission is fully compatible with X-linked recessive inheritance. As a hemizygous male, I-2 must have transmitted the trait to all of his four daughters. Three of them, II-1, II-2 and II-3, have inherited an additional defective allele from their mother and are thus homozygous, as evidenced by the presence of clinical symptoms. Therefore their mother I-1, who was a first cousin of her ichthyotic husband, must have been a heterozygous carrier. As expected, both sons of homozygotes II-1 and II-3 are affected, and so by chance are both sons of their heterozygous sister II-4. Subjects III-2, III-4, IV-2 and IV-7 are daughters of affected (homozygous) females or (hemizygous) males, respectively; as such, they are obligate heterozygotes. In addition, III-5, IV-1 and IV-4 are possible carriers.

It is evident from the above discussion that although gross and microscopic morphology together with genetic considerations tend to point to XRI they do not permit a diagnosis with certainty. However measuring the activity of steroid sulphatase in cultured skin fibroblasts from Cases II-1 and II-2 has enabled us to classify their ichthyosis unequivocally.

As first suggested by Jöbsis *et al.* (1976), XRI is related to the syndrome of placental steroid sulphatase (STS) deficiency. The latter is characterized clinically by failure of normal parturitional mechanisms, not infrequently necessitating a Caesarean section. In fact, this syndrome and XRI represent two different manifestations of generalized STS deficiency, which can be demonstrated in fibroblasts (Shapiro *et al.*, 1978a) and lymphocytes (Müller *et al.*, 1980a), and is inherited as an X-linked recessive trait. While seemingly all STS-deficient individuals have XRI, birth complications are known to occur in only some of them. In our family, only one out of seven pertinent pregnancies had to be terminated by Caesarean section.

Since STS activity is normal in other types of ichthyosis, including ADI and LI (Shapiro *et al.*, 1978b), a deficiency of the enzyme may be used as a biochemical marker to confirm the diagnosis of XRI. Moreover, as shown recently by Müller *et al.* (1980b), determination of STS activity can be employed successfully for heterozygote detection. In cultured fibroblasts, STS levels of normal females are about twice those of normal males while heterozygotes are consistently found in the low male range; this is due to non-inactivation of the STS locus which is now well established (Shapiro *et al.*, 1979; Müller *et al.*, 1980c). Cases II-1 and II-2 had nearly unmeasurable STS activities. Therefore they must be homozygotes carrying a double dose of this X-linked gene defect. This is compatible with the clinical findings and the pedigree. To our knowledge, this is the first report of clinically manifest X-linked recessive ichthyosis in women. Their phenotype, as expected, does not differ from that of men with XRI.

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Familial mandibuloacral dysplasia

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SUMMARY

A family is described in which two sisters and their two female fifth-cousins were cases of mandibuloacral dysplasia. This syndrome is characterized by mandibular hypoplasia, delayed cranial suture closure, dysplastic clavicles, abbreviated terminal club-shaped phalanges associated with acroosteolysis and atrophy of the skin over hands and feet. Furthermore, in this amily alopecia and loss of the lower teeth were noted.

We stress the importance of the cutaneous alterations and the possible existence of partial forms of the disease as in one of the patients presented.

Young *et al.* (1971) identified a new syndrome called mandibuloacral dysplasia. The main features of this syndrome are mandibular hypoplasia, delayed cranial suture closure, dysplastic clavicles, abbreviated terminal club-shaped phalanges associated with acroosteolysis and atrophy of the skin over hands and feet. Short stature, multiple wormian bones, prominent eyes and sharp nose are other common characteristics (Danks *et al.*, 1974; Welsh, 1975). Previously, patients with this condition had been mistakenly diagnosed as having Werner's syndrome (Cohen, Thurmon & Salvaggio, 1973), or cleidocranial dysplasia (Cavallazzi, Cremoncuri & Quadri, 1960) or acrogeria (Levi *et al.*, 1970). The condition is not described in dermatological texts although cutaneous manifestations include atrophy with mottled pigmentation and decreased fat deposits over acral regions.

We report a family in which four members showed this syndrome associated with progressive loss of lower teeth and alopecia.

CASE REPORT

Case 4 has been personally observed by the authors. She suffered from an incomplete form of this syndrome and was diagnosed correctly only when she informed us that her sister (case 3) and two female fifth-cousins (cases 1 and 2) were suffering from the same condition. Studying their clinical documents we recognized they were typical cases of mandibuloacral dysplasia.

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Case 1

In 1976 when she was 46 years old, N.E. weighed 38 kg and her height was 1·50 m. On examination she showed a thin face with prominent eyes and beaked nose. Her mandible was hypoplastic and her shoulders appeared narrow. Hands and feet were minute with short terminal phalanges. Over acral regions the skin was atrophic and areas of mottled hyperpigmentation gave the skin a poikilodermatous appearance. Subcutaneous fat was absent and veins and tendons were visible. The scalp hair was sparse and lustreless. The clinical history was uneventful except for recurrent arthralgia. Radiographic examinations showed diffuse osteoporosis of the skeleton. Her clavicles were hypoplastic with complete absence of the medial portions; also the 1st, 2nd and 4th ribs were hypoplastic and showed osteolytic areas. Hands and feet showed osteolysis of terminal phalanges with persistence of a small bone nucleolus. The long bones (humerus, femur, tibia) showed irregular thickening of the cortex. All cranial sutures appeared wide and the mandible was edentulous and showed a hypoplastic body and ramus. Endocrine tests showed no thyroid dysfunction. Her menses were normal. Her karyotype was normal (46 xx). No haematological or biochemical abnormality was found. Prolonged treatment with calcitonin reduced her pain but produced no radiological changes.

Case 2

N.M., N.E.'s sister, 30 years old in 1976, weighed 41 kg and her height was 1·50 m. She showed a facial resemblance to her sister and there were identical digital anomalies. On the limbs the skin was atrophic and the subcutaneous fat missing. By the time she was 14 years old she had lost all her lower teeth and then gradually she developed hair loss from the scalp. Joint stiffness was her greatest trouble and at intervals swelling and redness developed in single joints. Radiographic examinations demonstrated the same alterations as in her sister, chiefly wide cranial sutures, hypoplastic clavicles and mandible. No endocrine abnormalities were found. Her karyotype was 46 xx.

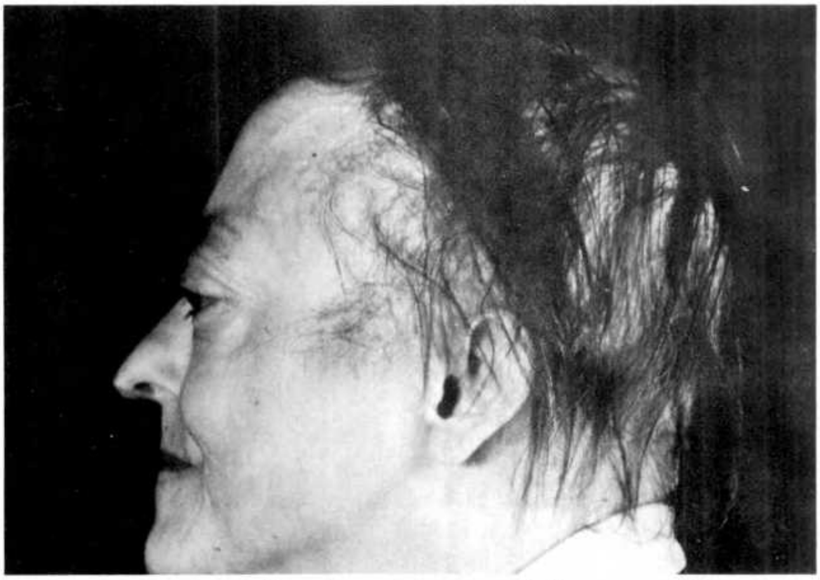

FIGURE 1. Case 4. Alopecia, prominent eyes and thin nose are evident.

Case 3

Me.L., our patient's sister, was a small woman with small hands and feet, looking older than her true age (37 years) because of her facies. A chest radiograph showed hypoplastic clavicles with complete absence of the medial portions. She also had lost her lower teeth and when she consulted a dentist she was recognized as having a hypoplastic mandible and persistently wide cranial sutures. Apart from this she had always enjoyed good health.

Case 4

Me.M. was a 40 year old woman who weighed 31 kg and her height was 1·40 m. She had a pinched bird-like facies with exophthalmos (Fig. 1). She had always had prominent eyes, but this worsened following inappropriate treatment with thyroid hormone. Her scalp hair was sparse and lustreless (Fig.1). Her hands and feet were small with club-shaped terminal phalanges and her fingers and toes looked like a tree-frog's leg (Fig.2); there the skin was atrophic and the subcutaneous tissue was absent. Her nails appeared overcurved like a parrot's beak, probably due to the underlying phalangeal abnormalities. Development had been normal until the age of 6 when gradually the limbs and the face acquired their abnormal features. Her menses began when she was 14 years old. By the age of 20 all her lower teeth had been lost while her upper teeth remained normal. Loss of scalp hair began when she was 20 years old and has been steadily progressive. She married and gave birth to a girl who is now 14 years old and absolutely normal. In 1973 she was hospitalized because of sideropenic anaemia. Acute migratory monoarthritis (pain, redness, swelling) and joint stiffness have greatly troubled her for years. No cause for this was identified and episodes of joint activity were controlled with small doses of aspirin. Radiographs of hands showed osteolysis of terminal phalanges with persistence of a small bone nucleolus. The clavicles appeared small but were complete; cranial sutures, especially the lambdoidal sutures, were wide; the edentulous mandible appeared substantially normal. A skin biopsy was performed on one arm. The epidermis showed a loss of

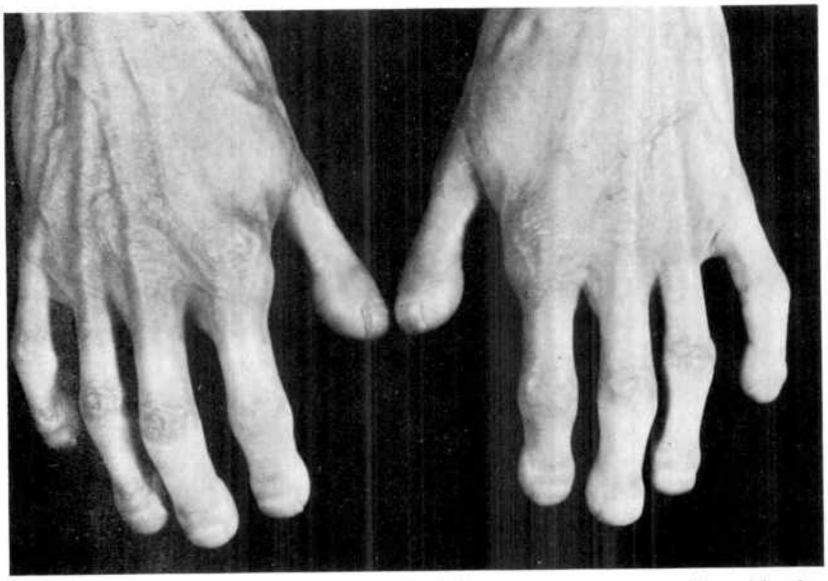

FIGURE 2. Case 4. Short club-shaped terminal phalanges; cutaneous atrophy with absence of subcutaneous fat produce a tree-frog appearance of hands.

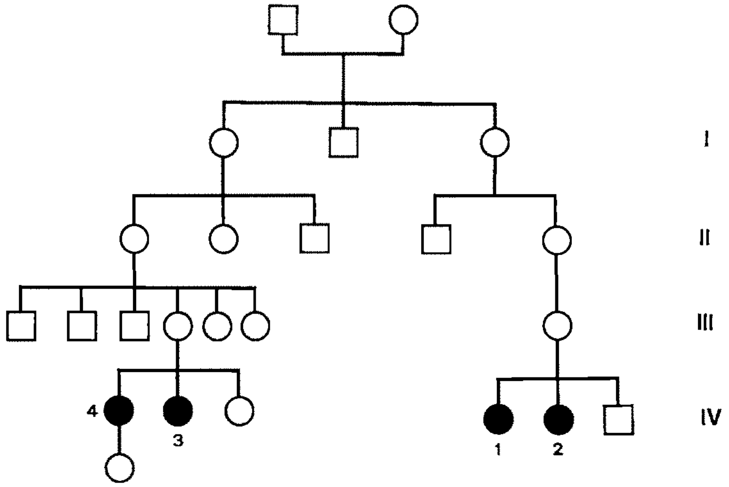

FIGURE 3. The pedigree made according to information obtained from members of this family. ○ Normal female □ Normal male.

rete pegs. The dermis was substantially normal and elastic fibres were well developed; subcutaneous fat was nearly absent.

DISCUSSION

According to our patient there were no other cases in this family (Fig.3). The condition appears to be inherited as a recessive and the link between the two pairs of affected sisters was their great-grandmothers, who were sisters.

Cutaneous changes are an important feature of this syndrome but in earlier reports they were not mentioned by the authors (Cavallazzi *et al.*, 1960; McKusick, 1972) even if they were evident from the published photographs. An important feature in this family was the shedding of scalp hair and alopecia appears to be part of the clinical spectrum of mandibuloacral dysplasia. We think this condition must be grouped with the syndromes in which premature ageing occurs (progeria, acrogeria, etc.). Indeed this new syndrome shows many features present in progeria and acrogeria (Gilkes *et al.*, 1974) and the cutaneous abnormalities are exactly the same in all these diseases.

The existence of incomplete cases of mandibuloacral dysplasia as in our patient (Case 4) (normal mandible and moderate involvement of clavicles) must be considered. Furthermore the case published by Levi *et al.* (1970) as acrogeria showed the clinical features and some of the bone malformations present in mandibuloacral dysplasia and for this reason it might be considered a defective form of this new syndrome.

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Comment

PAIN, ITCH AND ENDORPHINS

In the normal mammalian brain there are opiate receptors and naturally occurring peptides with opiate activity (Guillemin, 1977). These peptides known as enkephalins and endorphins act as neurotransmitters in the central nervous system (CNS) and are present in high concentrations in pain pathways (Snyder, 1977). The evidence that endorphins have a role in human pain comes largely from studies in which the action of endorphins has been blocked by naloxone. Naloxone is a pure opiate antagonist with no agonist activity: it has no detectable effect in normal man (Jasinski, Martin & Haertzen, 1967). However, naloxone can alter the subjective assessment of pain. In patients with dental pain naloxone causes hyperalgesia (Levine *et al.*, 1978a) and this appears to be due to the abolition of the placebo response (Levine, Gordon & Fields, 1978b). It therefore seems probable that the placebo response, at least in dental pain, may be mediated by the release of endorphins. At what level of the CNS these incoming pain impulses are modulated by an endorphin system is still unclear. However, the demonstration of an enkephalin system in the interneurones of the substantia gelatinosa of the dorsal horns (Hökfelt *et al.*, 1977; Jessell & Iversen, 1977) suggests that the incoming pain impulses may be modulated as they enter the dorsal horns of the spinal cord possibly by a 'gate control' mechanism such as that proposed by Melzack & Wall (1965).

There are several similarities between the physiology of itch and pain. Receptors for itch and pain appear to be spatially separated in the skin (Herndon, 1975). Stimuli which at low intensity induce itch at a higher intensity cause pain. Removal of the epidermis and upper dermis abolishes the capacity for itch but not for pain. Both pain and itch are transmitted to the spinal cord by small diameter (c) fibres (Zotterman, 1939) and are probably integrated at a common site in the dorsal horn of each spinal segment (Graham, Goodell & Wolff, 1951). These observations have prompted studies of the effect of naloxone on itch. In one patient, naloxone (0·8 mg) was reported to relieve the itching of cholestatic liver disease for about 2 h (Bernstein & Swift, 1979). However, in a larger placebo controlled study of patients with itching due to liver disease, a higher dose of naloxone (2 mg) did not uniformly reduce itching (Summerfield, 1980). An effect was only apparent when patients whose itch was reduced by a placebo (placebo responders) were separated from those whose itching was unchanged or worse after placebo (placebo non-responders). The placebo responders reported significantly greater itch after naloxone than after a placebo and this appeared to be due to the abolition of the placebo response. In contrast, placebo non-responders reported less itch after naloxone than after a placebo. Thus, naloxone also appeared to compete with an endorphin system that modulates the perception of itch. However, naloxone could either increase or decrease itching depending on the patient's response to a placebo. Although these results differed from those observed in dental pain, where the effect was limited to placebo responders (Levine *et al.*, 1978b), a bidirectional response to naloxone has

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been reported from a study of experimental pain (Buchsbaum, Davis & Bunney, 1977) and could be related to differences in naloxone dosage (Levine, Gordon & Fields, 1979). It is noteworthy that scratch, the usual consequence of itch, was not affected by naloxone (Summerfield, 1980). However, scratch may be a spinal reflex during sleep (Savin *et al.*, 1975) and it is possible that it is not modulated by the processes that mediate the perception of itch.

These preliminary results with itching now need to be confirmed and extended. The obvious questions that need answering are the dose/response relationships between naloxone and itch and the time course of this effect. The principal problems are those of experimental design. It is difficult to assess a subjective sensation such as itch although the analogue techniques developed for pain may be useful (Joyce *et al.*, 1975). The studies must also take account of the marked variability of the symptom and the pronounced placebo response in some patients. Answers to these questions will not only further our understanding of the physiology of itch but may also help untangle the complex relationships between itch and pain.

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Willan Library Bulletin

British Association of Dermatologists

WILLAN LIBRARY BULLETIN VI
1980

The Willan Library has enjoyed a successful year. Generous gifts from Professor Esteves, Dr and Mrs Robert Jackson, Dr Coleman Jacobson and Dr Bethel Solomons have made interesting additions to the Library. It has been a good year for purchases too; the rarer works on dermatology seldom come on the market, but this year the Library has been able to acquire almost all the works of Ernest Bazin. Bazin was of course one of the great figures of dermatology during the middle years of the nineteenth century and he made numerous important contributions for which he confidently gave himself full credit. During the decades from 1820 to 1860 dermatologists were preoccupied with problems of classification, or what passed as such, but which in fact reflected much more fundamental differences in approach. Bazin claimed credit for reconciling the two schools of thought, whose supporters were commonly known as the Willanists and the Alibertists. The controversy raged with a virulence which it is now difficult to appreciate.

Among the rarer books purchased during the year was an Essay by Prosper Baumès, of Lyon. In it he refers to this dominant controversy and summarizes his earlier works, one of which gave an account of his visits to the hospitals of London and Paris in 1834. This book is now exceedingly rare and no copy can be traced in any library in Britain. A microfilm copy was kindly provided by the Surgeon-General's Library in Washington. The Willan Library has now (1981) acquired a copy. Prosper Baumès forms the subject of this year's biobibliography. The accessions to the Library are listed at the end of the Bulletin.

The Willan Librarian will be glad to hear from readers who have old or rare works on dermatology which they are willing to offer to the Library. The Library is housed in the Willan Room of the Royal College of Physicians, London. Historians of dermatology wishing to visit the library should apply to the Willan Librarian.

ARTHUR ROOK
Addenbrooke's Hospital, Cambridge
Honorary Willan Librarian

PROSPER BAUMÈS, DERMATOLOGIST OF LYON 1791–1871 AND THE WILLAN-ALIBERT CONTROVERSY

Prosper Baumès (1) was born at Montpellier in 1791. He attended a college at Perpignan, and then taught mathematics there for a time. He continued to do so when he first started his medical studies at Perpignan Hospital. He next became a student at Montpellier and then at Lyon, where he graduated M.D. in 1823. Having failed to obtain a hospital appointment he started in practice in a poor quarter of Lyon, and achieved considerable success. In 1832 he was appointed Surgeon

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to the Hospice de l'Antiquaille, and it was at this point in his career that he visited the hospitals of Paris and London. His first publication, which appeared in the same year, was remote from dermatology. His obituarist suggests that his interest in flatulent dyspepsia was probably a personal one—Baumès, P. (1832) *Lettre sur les causes et effets de la présence des gazs ou vents dans les voies gastriques,* pp. 103. Paris.

Soon after his return from his travels Baumès made his first contribution to dermatology—Baumès, P. (1834) *Lettre d'un médecin de province à MM les Dermatophiles de Paris,* pp. 48. Paris.

In this pamphlet he somewhat aggressively asked his Paris colleagues what they had done to the broadly based and practical science established by Lorry. The following year he published his account of his visit to London, an interesting work which is considered in further detail below—(WL*) Baumès, P. (1835) *Aperçu médical des Hôpitaux de Londres.* Paris. (WL) Baumès, P. (1839) *Essai sur La Flaxion, appliquée à la considération théorique et pratique des Maladies de la Peau, ou Précis analytique sur ces maladies.* Paris.

In this monograph he made his first published attempt to introduce a rational approach to dermatology. He favoured abandoning the traditional terms of hybrid Greek and Latin origin and substituting vernacular terms. He recognized a number of 'special eruptions' such as scabies, zoster and favus, and a number of general patterns of eruption.

Despite his official appointment as Surgeon in Chief to the Antiquaille his beds were filled with dermatological and venereological cases. In venereology, referred to euphemistically by Baumès' obituarist as Ricord's speciality, he made important advances; he appreciated that by the time the primary chancre had developed in syphilis it was already a systemic disease. Many observations for which others have been given the credit were contained in his large text on venereology—Baumès, P. (1840) *Précis théorique et pratique sur les maladies vénériennes,* 2 vols, pp. 419, 580. Paris.

Baumès was a scholarly man of many talents. He was a violinist of recognized ability. His early mathematical training led him to demand from others, as from himself, an orderly and logical approach. As his obituarist expressed it, he combined the mind of a poet with the heart of an artist and the stomach of a dyspeptic, and was sometimes unpredictably moody and difficult. He acquired a large practice predominantly in dermatovenereology, but he was also a pioneer in the use of the laryngeal mirror. He ran courses on dermatology at the Antiquaille—Baumès, P. (1842) *Nouvelle Dermatologie, un précis théorique et pratique sur les Maladies de la Peau,* 2 vols, pp. 560, 622.

At the height of his reputation, in the 1840s, he left Lyon for the small country town of Lagnieu. Here he continued to keep in touch with the medical journals, but retired from practice apart from a few patients to whom he charged no fees. In 1851 he was elected mayor of Lagnieu. In his retirement he wrote his last book—Baumès, P. (1853) *Précis théorique et pratique sur les Diathèses,* Pp. 245. Paris. This work is said to have greatly influenced Bazin and Hardy.

Baumès died in 1871.

Baumès on London dermatology in 1832

Baumès opens his account of London Dermatology with a discussion of Bateman's 'Practical Synopsis', because although Bateman had died in 1820, the current (seventh) edition was edited by A. Todd Thomson and had appeared in 1827. Baumès criticises Willan's classification for taking no account of causes or of constitutional states. Bateman's interpretation of the views of Antoine Lorry led Baumès to wonder whether Bateman had ever actually read Lorry's works. He thought rather more highly of John Wilson, a forgotten disciple of Willan. Wilson in 'A practical treatise on Cutaneous Diseases' 1814, expressed, he thought, rational and 'truly medical views', but he accuses him of quoting Lorry's opinions without mentioning their source.

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* WL = Copy in the Willan Library.

Samuel Plumbe's 'Practical Treatise on Diseases of the Skin' (3rd Edition 1832) prompted him to wonder whether there had been any point in translating Biçhat's works into English, since Plumbe had completely ignored Biçhat's theories. Xavier Biçhat (1771–1802) was an outstanding pioneer in detailed anatomical studies. Jonathan Green, another disciple of Willan, and author of a 'Practical Compendium on Diseases of the Skin' 1875, was accused by Baumès of preparing a compilation merely to publicise his Medical Baths establishment.

In summing up the London dermatologists, 'Willanites to a man', Baumès concludes that 'any impartial physician' will agree that Alibert's first classification, despite its faults, best fulfils the requirements. 'In cutaneous pathology the study of the external features is of little importance. Only a classification based on causes is really acceptable. Until further observations have been accumulated and it is possible to determine the true significance of each form, it would be misleading to impose a classification which may be appropriate for natural history, but is not appropriate in medicine.' Baumès then suggests a very basic morphological classification into localized groups, which is in effect Willan simplified.

In the second half of his book Baumès discusses the treatment of venereal diseases. He found that all the London Hospitals accepted patients with these diseases. In reviewing the works of British authors on venereal diseases, he mentions that John Hunter's description of the chancre is ambiguous, and that in any case it had been clearly described by John of Vigo (1460–1520). He noted the almost universal enthusiasm for mercury: there was only one hospital in London not using mercury in primary syphilis. Liston at University College used antiphlogistic treatment. Baumès criticised William Wallace's 'Treatise on the Venereal Diseases' 1833: 'If one had set out to make the subject totally incomprehensible one could not have succeeded better.'

Baumès was clearly appalled by what he considered to be the abuse of mercury in London. It was generally accepted in many countries that mercury had its place in the management of some cases, but he deplored this wholesale reliance upon it. 'Let us hope that our London colleagues will eventually agree with us on this point.' He despaired of this coming about because, although there was no shortage of men of merit in London, the system of medical education lacked any solid foundation in 'physiological pathology'.

The Willanist-Alibertist controversy in retrospect

The remarkable renaissance of French medicine after the Revolution of 1789 made Paris the leading medical centre of the Western world for the first half of the nineteenth century. New and exciting ideas and approaches led to important discoveries in many branches of medicine. Baumès' criticism of medical teaching in London was fully justified. His criticism of the Willanists merely because they adopted this approach, was less so. Alibert, a characteristic product of the revolution in French medicine, was highly intelligent and highly imaginative, as was his approach to dermatology, but he was teaching dermatologists to run before they had learned to walk. Willan's approach was the slow, step by step analysis of what we now call physical signs. It was Laurent Théodore Biett (1781–1840), Alibert's senior assistant, who, after attending Bateman's clinics in London in 1816, introduced the duller, plodding but more logical Willanist approach to France. From about 1826 there were these two opposing schools of dermatology in France. Cazenave, who succeeded Alibert, Devergie, who succeeded Biett, and Rayer and Vidal were all Willanists. Alibert's approach involved a far greater element of speculation, but he was an acute clinical observer and he described mycosis fungoides, cheloid, acrodynia, pityriasis rubra pilaris, and seborrhoea, and he recognized the role of 'uterine troubles' in some case of acne (2).

Alibert's clinical observations were widely accepted in Britain but his classification and his elaborate new nomenclature were not. One of his few British disciples was John Paget (1808–1892), who was M.D. Edinburgh 1831. He worked at the Hôpital St-Louis with Alibert and his essay, which won the Alibert Prize in 1833, gives the best contemporary account in English of the Alibert doctrines (3). However Paget did not continue to practise. He married a Hungarian baroness and went to Hungary to manage her estates.

Between 1826 and the 1860s most dermatological authors devoted what now seems a disproportionate amount of space to the Willan-Alibert controversy. It was resolved, as the study of Baumès' writings illustrates very effectively, not by the efforts or inspiration of Bazin or of any other individual, but by the gradual accumulation of scientific observations, for example the discovery of the dermatophytes, which gradually replaced speculation, and the acceptance of a pragmatic critical approach to those many dermatoses, the cause of which remains mysterious.

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1. Diday, M. (1874) Note historique sur le Docteur P. Baumès. *Lyon Médical*, 25, 396.
2. Brodier, L. (1923) J.-L. Alibert, pp. 25–26. Maloine, Paris.
3. Paget, J. (1832–3) *Lancet*, ii, 490, 524, 566; *Lancet*, i, 262, 303.

ACCESSIONS TO THE WILLAN LIBRARY 1980

Accessions to the library during the year include.

Bernardino Antonio Gomes (1820) *Ensaio Dermosographico ou Succinta e Systematica Descripção das Doenças Cutaneas, Conforme os Principios e Observações dos Doutores Willan, E. Bateman, com Indicação dos Respectivos Remedios Aconselhados por Estes Celebres Authores, e Alguns Outros*. Pp. XII, XXV–171. Lisbon, Academy of Medicine (presented by Professor J. A. Esteves of Lisbon).

The author is regarded as the founder of dermatology in Portugal. He was a Court Physician and he dedicated this book to The Princess Royal of Portugal, Brazil and Algeciras.

H. Jausion and F. Pagès (1933) *Les Maladies de Lumière et Leur Traitement*. Pp. VI–204. Masson, Paris (presented by Dr Coleman Jacobson). Jausion, who was professor of the Val de Grace, was an important pioneer in the scientific investigation of the photodermatoses.

W. Frieboes (1921) *Grundriss der Histopathologie der Hautkrankheiten*. Pp. VIII–208. Vogel, Leipzig (presented by Dr Coleman Jacobson).

Frieboes (1880–1945) was at that time Professor of Dermatology at Rostock. This is a short, clearly written, well-illustrated introduction to cutaneous histopathology. Frieboes was later Professor at the Charité Hospital in Berlin.

E. Bazin (1859) *Leçons Théoriques et Cliniques sur Les Syphilides Considérées en Elles-Mêmes et dans Leurs Rapports avec les Éruptions Dartreuses, Scrofuleuses et Parasitaires*. Pp. X–224. A. Delahaye, Paris (purchased).

bound with

E. Bazin (1853) *Recherches sur la Nature et le Traitement des Teignes*. Pp. 152.

and

E. Bazin (1858) *Leçons Théoriques et Cliniques sur les Affections Cutanées Parasitaires*. Pp. XVI–236.

E. Bazin (1862) *Leçons Théoriques et Cliniques sur les Affections Cutanées Artificielles et sur La Lèpre, les Diathèses, le Purpura, les Difformités de la Peau, etc*. Pp. XI–499. A. Delahaye, Paris (purchased).

E. Bazin (1862) *Leçons Théoriques et Cliniques sur les Affections Génériques de la Peau*. Pp. XII–416. A. Delahaye, Paris (purchased).

E. Bazin (1865) *Leçons Thóriques et Cliniques sur les Affections Génériques de la Peau.* Vol. 2. Pp. VIII–451. A. Delahaye, Paris (purchased).

Ernest Bazin (1807–1878) was a physician to the Hôpital St-Louis in Paris. He made numerous contributions to dermatology. Most of his writings were prepared from lecture notes by one or other of his pupils and revised by Bazin himself. His 1853 monograph on the nature and treatment of ringworm is of particular interest as he accepted the significant and causative role of dermatophytes in ringworm at a time when most dermatologists refused to do so. His 'Leçons' cover the whole field of dermatology and give more insight into the causal concepts of diseases than does a formal textbook, for the speaker is often thinking aloud. Bazin was outspoken and dogmatic and supercilious to those who did not share his views.

H. Hallopeau and L. E. Leredde (1900) *Traité Pratique de Dermatologie.* Pp. VIII–992. Baillière, Paris (purchased).

Henri Hallopeau (1842–1919). He was a physician to the Hôpital St-Louis, where he worked with Besnier. His textbook was the standard work in French for two decades. Leredde was director of the laboratories at the Hôpital St-Louis.

F. Hirsch (1956) *Das Haar des Menschen.* Pp. 301. Haug, Ulm-Donau (purchased).

This monograph, little known in Britain, presents a chemist's well-documented review of the literature on human hair, with emphasis on physical and chemical properties, but it covers other aspects, including the clinical. Hirsch was particularly interested in keratin.

J. S. Huxley and G. R. de Beer (1934) *The Elements of Experimental Embryology.* Pp. XIII–514. Cambridge University Press (presented by Dr and Mrs Robert Jackson in memory of Dr Ian Whimster).

J. Hilton (1880) *Rest and Pain: a Course of Lectures on the Influence of Mechanical and Physiological Rest in the Treatment of Accidents and Surgical Diseases, and the Diagnostic Value of Pain.* 3rd Edition. Pp. XV–511. Bell, London (presented by Dr and Mrs Robert Jackson in memory of Dr Ian Whimster).

These two classics complete the collection of books presented by Dr and Mrs Robert Jackson in memory of Dr Ian Whimster. They were chosen because Dr Whimster considered that they had strongly influenced his approach to medicine in general and to research in particular. All the works are to some extent timeless. They can still be read with pleasure and they continue to stimulate.

Tilbury Fox (1877) *Skin Diseases: Their Description, Pathology, Diagnosis, and Treatment.* 2nd American Edition, from 3rd London Edition, revised and enlarged. Pp. XIV–532. Wood, New York (purchased).

This edition of Tilbury Fox's outstanding textbook was edited, as was the first American edition, by M. H. Henry of New York, founder of the *Journal of Dermatology.* In his preface Henry writes: 'It is, I think, the very best work of skin diseases ever offered to the American student and practitioner of medicine.'

British Journal of Dermatology (1981) **105**, 733.

Book Reviews

Microbiology of Human Skin (Noble and Somerville). By W.C.NOBLE (1981) London: Lloyd-Luke. 2nd Edition. Pp. 433. Price £25.00.

The first edition of this book, published in 1974, has become accepted as a standard work. It includes all kinds of microorganism—bacteria, fungi, viruses, mycoplasmas, parasites—and describes these as transients, commensals and pathogens, their interrelation and their dependence on the underlying structure and activity of the skin. The ecology of the normal flora is described, and the scientific basis of pathological states, with sufficient clinical information. The role of the skin as a carriage site for microorganisms that cause infection elsewhere is discussed. This first edition has been used as a reference book over the years and has hardly ever failed to provide information and indicate further sources in the literature.

The new edition is welcome as a necessary replacement. Despite omission of certain material and references, it is almost a third longer, for there have been many developments and new observations in this field. These have not always made the subject clearer or less complex, but the new material is completely integrated and critically assessed.

Among additions, Professor Noble had included a brief account of his own interesting work on transfer of antibiotic resistance from coagulase-negative staphylococci to *Staphylococcus aureus* on the skin, and also particularly helpful sections on the controversial taxonomy of coryneforms and the *Micrococcaceae*. Reading the new edition through, this reviewer has encountered many pieces of useful information and correctives to prevailing misconceptions, enjoyed the humorous asides and the intriguing entries on such subjects as the role of bacteria in the production of body odours, and the microbiology of beards, and been made to appreciate how much remains to be learnt about the skin and its microflora.

D.C.E.SPELLER

Geriatric Dermatology. By G.W.KORTING. Translated by W.CURTH and H.O.CURTH (1980) Philadelphia: W.B.Saunders. Pp. 194. Colour plates 141. Price £25.00.

Dermatology is dermatology as far as I am concerned, and any attempts to fragment it into paediatric, adolescent, geriatric or dermatoses of the 37½-year-old meet with a certain lack of sympathy. Dr Korting proves my point by providing us with what is essentially a small text book of dermatology, albeit with a geriatric slant, but containing large sections on such topics as hereditary and congenital disease. He has compounded his problems by somehow managing to insert 600 references into a mere 160 pages of text, leaving little space for coherent transmission of ideas. The translators have spared us several reports of onychogryphosis in the red deer, which appeared in German hunting journals, but they have spared us little of the heavy Germanic phraseology, and appear to have made little effort to Anglicise the diagnostic labellings. Morbus Paltauf-Sternberg for example should translate into Hodgkin's lymphoma, and pemphigus of the elderly, or parapemphigus, should surely now be called pemphigoid.

The photography is excellent, but I fear that it would not justify the expenditure of £25.00.

F.A.IVE

British Journal of Dermatology (1981) **105**, 734.

News and Notices

Cell Proliferation in Psoriasis

An international workshop devoted to the analysis of methodology and kinetic and biochemical mechanisms in proliferative skin disease, and to the exploitation of cell kinetics in the chemotherapy of psoriasis, will be held in Oxford, 5–7 April 1982. Further details are available from Professor N.A.Wright, Department of Histopathology, Royal Postgraduate Medical School, Ducane Road, London W12 0HS.

The Royal College of Physicians, Edinburgh

Dr M.J.Deakin has recently been elected F.R.C.P. (Edinburgh).

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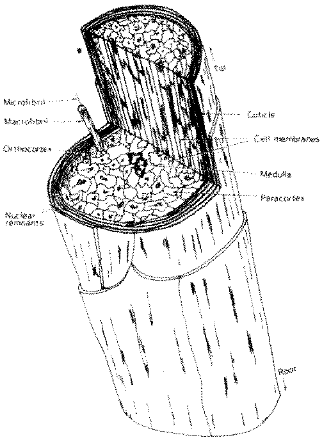

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Biochemistry of Normal and Abnormal Epidermal Differentiation

Proceedings of the U.S.-Japan Seminar,
Boyne Mountain Lodge, Boyne Falls, Michigan,
July/August 1979

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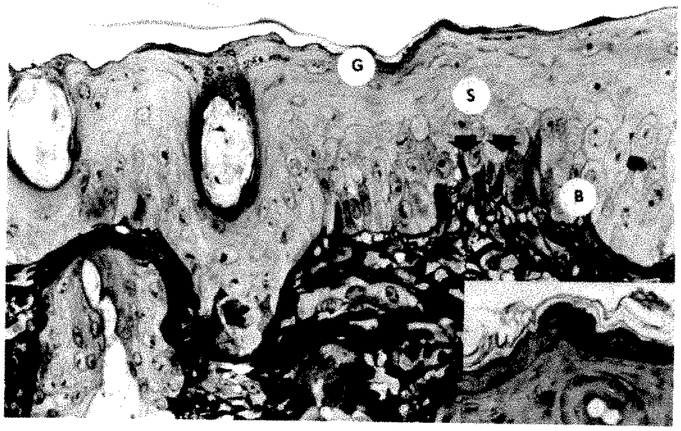

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microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
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